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Award Number: W81XWH-11-1-0376

TITLE: Comprehensive Population-Specific Marker Panel for Early Prostate Cancer  
Diagnostics and Risk Assessment

PRINCIPAL INVESTIGATOR: Ganna Chornokur

CONTRACTING ORGANIZATION: Moffitt Cancer Center  
Tampa, FL 33612

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14. ABSTRACT The overall scope of the proposed postdoctoral training project is to provide protected time for the primary investigator (PI) to obtain a comprehensive training in the field of prostate cancer health disparity. Within the scope of this grant, a comprehensive training consists of the two main parts; a training part and a research part. For the training part, PI attended relevant seminars, conferences, classes, and wrote manuscripts and grants. For the research part, we hypothesized that a combination panel (DETECT) of genetic biomechanical, socio-cultural and lifestyle population specific biomarkers and factors will provided a valuable PCa screening and risk assessment tool. The PI genotyped 528 DNA samples obtained from African American and European American men with prostate cancer controls. It was found that two SNPs were statistically significantly associated with prostate cancer risk associated with one of the SNPs in African American men was found in obese men only; if was not seen in either non-obese African American men or European American men regardless of their body mass. The PI has proposed a concept of the increased risk in obese AAM for which the NIH R21 was submitted on 11/2012. The grant was scored in the 30 <sup>th</sup> percentile and will be resubmitted on 07/2013. In addition, the PI has genotyped the samples for the androgen receptor gene CAG repeat length, and found the connection with obesity of African American men. Investigations of these topics are on going					
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## Introduction

The overall scope of the proposed 2-year postdoctoral training project is to provide protected time for the Primary Investigator (PI) to obtain a comprehensive training in the field of prostate cancer health disparity. The PI is a postdoctoral scientist / young investigator whose long-term career goal is to contribute to the resolution of cancer-related health disparities by conducting innovative, high-impact research as an independent investigator with competitive, peer-reviewed funding. PI aims to achieve her long-term career goal through an integrated 2-year research and training program in the comprehensive H. Lee Moffitt Cancer Center and Research Institute, with appropriate guidance of an experienced Mentor, Dr. Nagi Kumar, who is a nationally recognized expert in the chemoprevention of cancer and is a leader in health disparities research, and the team of Co-mentors (Drs. Park and Phelan). Within the scope of this grant, a comprehensive training agenda has been proposed; it consists of the two main parts: a training part and a research part (supplemental project). Specific progress on each of those parts is outlined in the body of this annual report. It is resumed with the “Key Research Accomplishments” and “Reportable Outcomes” sections that briefly summarize the main reportable outcomes emanating from this work.

## BODY

**A. Training Program.** The specific activities of the PI’s career development plan are provided below.

***1. Structured mentoring program coordinated by PI/primary mentor and co-mentor(s).***

For the duration of this award (years 1 and 2), Dr. Chornokur has been maintaining weekly and/or bi-weekly one hour meetings with her primary mentor, Dr. Nagi Kumar, and co-mentors, Drs. Catherine Phelan and Jong Park. This schedule will remain in effect for the period of approved yearly no-cost extension.

***2. Gain proficiency and a better understanding of science used to develop and implement primary prevention intervention in minority populations.***

- During the years 1 and 2 of award, this task is being accomplished by: 1. regular meetings with the Mentoring team as described above; 2. conducting extensive bi-annual literature searches on the topics of chemoprevention of prostate cancer, prostate cancer racial health disparity, and other relevant literature (community outreach programs, for example); 3. being involved in the relevant research projects at the Moffitt Cancer Center (please see Table 1); 4. attending the relevant talks, ground rounds and seminars at Moffitt and USF (please see task 7); and 5. extensive networking (please see task 9). This comprehensive systematic approach has allowed Dr Chornokur to develop and submit three peer-reviewed grants (please see the task 6) and publish 6 peer-reviewed manuscripts with additional one currently undergoing peer-review (please see the task 8), and one being in preparation. The approved no-cost extension will likely yield additional deliverables including the grant proposal(s) and manuscripts.

***3. Gain hands-on research experience in the implementation and conduct of research studies.***

These activities has been going on during both the years 1 and 2 of award. This schedule will remain in effect for the duration of the approved yearly no-cost extension.

- **Under the mentorship of Dr Phelan, the PI is currently involved in the three NIH and DoD funded projects** (aside from her own research study): 1. PC050873 (Phelan PI): Prostate Cancer in African-American Men: Serum Biomarkers for Early Detection Using Nanoparticles; 2. 1R01 CA149429-01 (Phelan PI): The Mitochondrial Genome and Ovarian Cancer Risk; and 3. 5 U19 CA148112-02 (Sellers PI): Follow-up of Ovarian Cancer Genetic Association and Interaction Studies (FOCI). **The PI is also involved in the following NIH funded clinical trial under the mentorship of Dr. Kumar:** R01 CA12060-01A1 (Kumar PI): Phase II, Randomized, Double-blind, Multi-centered Study of Polyphenon E in Men with High-grade Prostatic Intraepithelial Neoplasia (HGPIN) and Atypical Small Acinar Proliferation (ASAP). The summary of these projects, along with specific PI’s activities, are shown in the Table 1.



Table 1. Research projects that the PI is currently involved with through her Mentors.

Project	Primary hypothesis, goals and/or objectives	PI's involvement, tasks etc
PC050873 Prostate Cancer in African-American Men: Serum Biomarkers for Early Detection Using Nanoparticles	In this study our objective is to set up a prostate cancer case-control study in order to develop new serum biomarkers for early detection of prostate cancer using Nanotechnology. It also sought to investigate whether any bio-behavioral risk factors are associated with prostate cancer in African American men.	The PI was involved in this study while doing her PhD work in Biomedical Engineering at the University of South Florida. The study is now closed for accrual, however, the data analysis and research dissemination continue. Specifically, under the mentorship of Dr Phelan, the PI collaborated with a statistician to investigate known bio-behavioral risk factors of prostate cancer, along with PSA levels, and risk of prostate cancer in African American / Black men. This work has generated intriguing pilot results that were presented by the PI as a poster at several relevant high-impact scientific meetings. The PI has also prepared a manuscript entitled "Risk Factors for Prostate Cancer in African American Men" that is now undergoing final revisions. This project, that is highly relevant to the PI's training and her own research work, provides plethora of vital skills needed to conduct an interdisciplinary health disparity research in prostate cancer. The latter includes review and understanding of the most up-to-date literature in the field, skills necessary to perform epidemiological and statistical analyses, and – the most importantly – understanding and interpretation of the findings. These skills will lay the foundation for the PI to start developing her own intervention and independent research in prostate cancer health disparity in the year 2 (as outlined in the task 5 of this award).
1R01 CA149429-01 The Mitochondrial Genome and Ovarian Cancer Risk	It was hypothesized that that inherited variation in mitochondrial-related genes is associated with ovarian cancer risk. The objective is to more comprehensively investigate the contribution of mitochondrial genome variation to ovarian cancer risk.	In this award, the PI is actively involved in the pathways and genes/SNPs selection, as well as analysis interpretation and dissemination. This includes extensive literature search on the topic, review of the molecular and biochemical mechanisms that may play a role in the development of ovarian cancer, and understanding the statistical analyses involved in the data management. Importantly, the PI is exposed to the opportunities and challenges of the multicenter, multi-institution, collaborative work that involves extraordinary big sample size. These experiences are vital for an independent health disparity researcher. In addition, the PI obtains basic understanding of ovarian cancer research through this project. While not explicitly health disparity related, this project may serve as a foundation for the PI's own future work in ovarian cancer health disparity.
5 U19 CA148112-02 Follow-up of Ovarian Cancer Genetic Association and Interaction Studies (FOCI)	Specific aims of this project include evaluating the role of candidate genes at susceptibility loci in ovarian cancer; determining the functional significance of top candidate SNPs; and performing detailed functional characterization of candidate genes and SNPs.	PI's involvement into this multicenter, multi-institution high-impact study is similar to the one described above (CA149429-01). However, it allows for an even broader understanding of all ovarian cancer research, mitochondria related or not. PI attends the regular monthly-scheduled conference calls where the findings, challenges, manuscripts and conference presentations, future funding opportunities and current progress are being systematically discussed. Similarly to CA149429-01, this work is not disparity-related, however, it exposes the PI to a unique research experience that is vital to a transdisciplinary health disparity researcher and would have been hard to impossible to obtain elsewhere.
R01 CA12060-01A1 Phase II, Randomized, Double-blind, Multi-centered Study of Polyphenon E in Men with High-grade Prostatic Intraepithelial Neoplasia (HGPIN) and	Men with HGPIN who receive Polyphenon E containing 400mg of EGCG per day for 12 months will significantly decrease progression to CaP compared with men with HGPIN who	PI attends the monthly research meetings where specific research-related progress is being discussed. PI is aware of the recruitment challenges and is involved in the interventions aimed at increasing the recruitment rates. In addition, the PI will shadow the project coordinator when she recruits and/or follows up new or existing patients. The PI is using this trial as an opportunity to learn how to conceive and implement a chemopreventive intervention in prostate cancer.

Atypical Small Acinar Proliferation (ASAP)	take placebo.	
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#### **4. Initiate and complete a research supplement project.**

- The Institutional Review Board (IRB) and Scientific Review Committee (SRC) approvals have been obtained by the PI. The copies of both approvals are attached in the appendices 1 and 2, respectively. At the end of year #1, specific aim 1 has been completed. At the end of year #2, specific aims 2 and 3 have been partially completed, pending full completion during the approved one year no cost extension. The data analysis, manuscript, presentation and grant writing will continue through the year of approved no cost extension. Please see the section "Research plan" for more details.

#### **5. To develop interventions and independent research questions based on the results from research study completed.**

- Based on the data obtained within the scope of this training grant, we hypothesized that select genetic variation, when combined with the environment of obesity, influences PCa risk by impacting key cellular processes relevant to the tumor biology. In the year 2 of this award, the PI, with the guidance of her Mentors, has developed a methodological pilot high-risk high-gain project to test that hypothesis. Please see the R21 details for additional information on the proposed study. The resubmission of the project and all related activities will continue during the approved no cost extension.

#### **6. Gain extensive grant writing experience.**

- As a part of her grant writing training, the PI has attended an NIH-organized grant writing workshop that was held during the annual AACR meeting (May 31, 2012, Chicago IL). She has also attended several scientific writing/ grant writing seminars (please see task 7, items B,J,K,L).

- During the year 1, PI has submitted the following two extramural peer-reviewed research grants: an NIH R03 on 02/2012 (1R03CA172753-01: Comparative race-specific chemopreventive effects of curcumin in prostate cancer), and a DoD hypothesis development award on 06/2012 (PC120156: Comparative race-specific chemopreventive effects of curcumin in prostate cancer). Specific aims page for the aforementioned grants is shown in the appendix 3. Although those grants were not funded, they nonetheless provided useful grant writing experience.

- During the year 2, PI has submitted an NIH R21 grant on 11/2012 in response to PAR12-094 (1R21CA179659-01: Obesity and prostate cancer risk in Black and White men: a functional approach). The proposal was scored in the 30<sup>th</sup> percentile with the priority score of 3.9. The PI is currently preparing a resubmission due 07/16/2013. Specific aims page for the aforementioned grant, as well as the summary statement, are shown in the appendix 4.

#### **7. To attend research and educational meetings.**

- PI is an associate member at the American Association for Cancer Research (AACR).

- During the year 1, PI has attended (and presented her work, where indicated) at the following relevant research and educational meetings.

1. Joining FORCES Against Hereditary Cancer Conference (June 2011, Orlando FL). The poster presentation entitled "Risk of Gastrointestinal Cancers in Female BRCA1 and BRCA2 Mutation Carriers".

2. Center for Equal Health Strategic Planning Retreat Meeting (September 2011).

3. Annual AACR meeting (March-April 2012, Chicago IL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".

4. The First Florida Prostate Cancer Research Symposium (May 4-5 2012, Orlando FL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".

5. Moffitt's 2012 Scientific Symposium (May 10 2012, Tampa FL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".

- During the year 2, PI has attended (and presented her work, where indicated) at the following relevant research and educational meetings.

6. The Second Florida Prostate Cancer Research Symposium (March 2013, Orlando FL). The poster presentation entitled “Androgen Receptor CAG Repeat Length and Association with Prostate Cancer Risk in Obese and Non-Obese African American men”.

7. AACR Annual Meeting (04, 2013): Variation in transmembrane transport genes influence epithelial ovarian cancer risk and histopathologic subtype (poster presentation). Authors: Chornokur G; Tyrer J; Lin H-Y; Han G; Qu X; Chen Z; Tsai Y-Y; Goode EL; Cunningham JM; Iversen E; Ramus S; Berchuck A; Schildkraut JM; Monteiro A; Gayther S; Narod SA; Pharoah P; Sellers TA; Phelan CM.

8. NIH “2012 Science of Eliminating Health Disparities Summit” (12, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation). Authors: Chornokur G; Amankwah EK; Park JY; Phelan CM; Kumar NB.

9. AACR “The Science of Health Disparities” Conference (10, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation). Authors: Chornokur G; Amankwah EK; Park JY; Phelan CM; Kumar NB.

The PI has received a competitive Scholar-in-training Award to cover the travel/attendance cost for this meeting.

The poster copies are included in the appendices 5-9. Presentations will continue through the approved no cost extension year.

PI is also attending relevant scientific talks, ground rounds and seminars that occur at Moffitt and/or USF. Select, the most relevant presentations are shown below.

A. Why Culture Matter in Eliminating Health Disparities, Collins Airhihenbuwa, PhD, June 3, 2011.

B. American Cancer Society-Funding Opportunities for Research and Training. Charles L. Saxe, PhD. Moffitt Cancer Center. August 1, 2011.

C. Fishers of Med: Evaluating a Digital Training Curriculum, Brian Rivers, PhD, MPH. August 31, 2011.

D. The Skin You're In: Making Progress in Eliminating Health Inequalities, Thomas LaVeist, PhD. November 4, 2011.

E. Medically Underserved Areas and Patient Navigation, Elizabeth Calhoun, Med, PhD. January 19, 2012.

F. A Biocultural Approach to Racial Disparities in Health: Genetics, Epigenetics, and the Biological Embedding of Psychosocial Stress, Amy Non, PhD. Candidate for Faculty Position in Biological Anthropology Seminar. January 23, 2012.

G. Minority Enrollment in Clinical Trials: Challenges and Best Practices, Mona N. Fouad, MD, MPH. February 13, 2012.

H. Participatory Research and Evaluation: Case for Building Practice-Based Evidence, Joseph Telfair, DrPH, MSW, MPH. Center for Equal Health External Advisory Board Meeting, February 16, 2012.

I. Planning and Implementing Lay Health Worker Interventions for Cancer Control: a Systematic Process to Increase Program Outcomes, Maria Fernandez, PhD. March 9, 2012.

J. Elements of Basic Science Grant Writing: A physician-scientist View, Eduardo Sotomayor, MD. MPDA Grant Writing Seminar. January 12, 2012.

K. Manuscript writing workshop: The Introduction, Methods, Results, and Discussion (IMRaD) Format, Jane D. Carver, PhD, MS, MPH. MPDA Career Development Seminar. February 21, 2012.

L. Enhancing Your Success as a Published Author, Jane D. Carver, PhD, MS, MPH. Cancer Epidemiology Career Development Seminar. March 21, 2012.

M. Socioeconomic Status and Tobacco Related Disparities. David Wetter, PhD, MS. May 11, 2012

N. Ethical Cancer Research & Uninformed Consent: The Henrietta Lacks Story. Organized by Moffitt Diversity Department. May 23, 2012.

**O. Past Experiences – Lessons Learned (SELECT, CARET, Retinoids vs. Statins, COX-2 inhibitors): What Went Wrong? Where Do We Go From Here?** Ernest Hawk, MD, PhD. May 29, 2012.

Additionally, as required by her work, the PI maintains the Human Subjects (certification updated on 04/2012), Biosafety (04/2012) and Mandatory Moffitt education (05/2012) tests and trainings up to date.

**8. *Scientific writing and research dissemination.***

- The following peer-reviewed research articles have been published or accepted for publication during the year 1 of this award (06/2011 – 06/2012):
  1. Shahnjayla Connors, Ganna Chornokur, Nagi Kumar. New Insights to the Mechanisms of Green Tea Catechins in the Chemoprevention of Prostate Cancer. *Nutrition and cancer*. 11/2011; 64(1):4-22; PMID: 22098273
  2. Kumar NB, Crocker, T, Smith T, Pow-Sang, J, Spiess, PE, Connors, SK, Chornokur, G, Iravani Dickinson, S, Williams, CR, Salup, R, Bai, W. Prostate Cancer Chemoprevention targeting High Risk Populations: Model for trial design and outcome measures. *J Cancer Sci Ther*. 2012; PMID: 22422102
  3. T.G. Kryshchak L.V. Borkovska O. Kolomys N.O. Korsunskaya V.V. Strelchuk, L.P. Germash, K.Yu. Pechers'ka, G. Chornokur, S.S. Ostapenko, C.M. Phelan, O.L. Stroyuk. The effect of bio-conjugation on aging of the photoluminescence in CdSeTe-ZnS core-shell quantum dots. *Superlattices and Microstructures* 51(3) 2012, 353–62
  4. Manju Venugopal, Sunil K. Arya, Ganna Chornokur, Shekhar Bhansali. A Realtime and Continuous Assessment of Cortisol in ISF Using Electrochemical Impedance Spectroscopy // *Sens Actuators A Phys*. 2011 1;172(1):154-160. PMID: 22163154
- The following peer-reviewed research articles have been published or accepted for publication during the year 2 of this award (07/2012 – 06/2013):
  5. **Chornokur G.**, Kumar NB. Prostate cancer chemoprevention in men of African descent: current state of the art and opportunities for future research. *Cancer Causes and Control* 2013 [in press]. DOI: 10.1007/s10552-013-0241-x. PMID: 23737026
  6. Kumar N, **Chornokur G** (2012) Molecular Targeted Therapies Using Botanicals for Prostate Cancer Chemoprevention. *Transl Med S2*: 005. doi:10.4172/2161-1025.S2-005
  7. **Chornokur G**; Han G; Tanner R; Lin H-Y; Green LB, Pow-Sang J; Phelan CM. High Grade Prostate Intraepithelial Neoplasia (PIN) is a PSA-independent risk factor for prostate cancer in African American men: results from a pilot study. *Cancer Letters* 331(2): 154-7, 2013. PMID: 23268329
  8. **Chornokur G**, Amankwah EK, Schildkraut JM, Phelan CM. Global ovarian cancer health disparities. *Gyn Oncol* 121(1): 358-64, 2013. PMID: 23266352
- The following research articles are currently being peer-reviewed:
  9. **Chornokur G**, Amankwah EK, Davis SN, Phelan CM, Park JY, Pow-Sang J, Kumar NB. Variation in *HNF1B* and obesity may influence prostate cancer risk in African American men: a pilot study. Submitted to “Cancer Prevention research”, 06/2013. [included in the appendix 10]
  10. Phelan CM, Iqbal J, Lubinski J, Ragone A, Cybulski C, Lynch HT, Moller P, Ghadirian P, Foulkes WD, Armel S, Neuhausen SL, Senter L, Singer CF, Kim-Sing C, Tung N, Llacuachqui M, **Chornokur G**, Ping S, Narod SA and the Hereditary Breast Cancer Study Group. Incidence of colorectal cancer in

*BRCA1* and *BRCA2* mutation carriers: Results from a follow-up study. Submitted to Br. J. Cancer, 05/2013. [included in the appendix 11]

- The following research article is currently in preparation:

10. **Chornokur G**, Amankwah EK, Lin HY .... and Phelan CM on behalf of OCAC. Cellular Transport Gene Variants Are Associated With Susceptibility to Epithelial Ovarian Cancer Risk and Histopathologic Subtypes. [included in the appendix 12]

Please see the section 7 for the list of attended meetings and poster presentations.

**9. *Interactions with established scientists, networking, and peer linkages.***

- During the years 1 and 2 of award, this training activity is being achieved through: **1.** working with collaborators (inside and outside of the mentoring team) to write joint grant submissions; **2.** contributing to manuscripts written by other scientists; **3.** networking and learning from peers and colleagues at the research meetings; **4.** participating in the USF/Moffitt Center for Equal Health; and **5.** attending the “networking with experts” lunches that follow bi-monthly ground rounds at Moffitt. These activities will continue during the approved yearly no cost extension.

**10. *Additionally, as requested by the peer-review committee,*** Dr. Chornokur has audited the “Cancer epidemiology” course at USF that was taught by two Moffitt Cancer Epidemiology Assistant Members. She has also completed the 5-weeks “Biostatistics 101” course (certificate awarded), designed and taught by the Moffitt Biostatistical core (both courses during the year 1 of award). During the year 2 of award, she has audited the “Cancer genetics” course (Spring 2013) taught by Dr. Alvaro Monteiro who is a Senior Member at Moffitt, as well as other Moffitt faculty.

**11. *Deliverables and Evaluation.*** *PI will be evaluated annually by Mentor, co-Mentor and other faculty. Annual evaluations will assist the Mentor in refining this training program.*

Successful annual evaluation is a part of this training grant, as well as a requirement a Moffitt Cancer Center sets for every postdoctoral scientist. This extensive evaluation process includes a rating of the postdoc’s behavioral criteria (teamwork, responsibility, adaptability etc), as well as professional accomplishments and progress within the past year. It also includes an outline of the major professional goals and career targets set for the next year. These goals are set up by a postdoc herself, and approved (with modifications, if needed) by her Mentor(s). To comply with this requirement, PI was evaluated by the team of her mentors during the year 1 in December-January 2011/2012 (the PI’s anniversary date at Moffitt). Based on this evaluation, PI has gotten an overall score of 4.2 out of 5 that corresponds to the “Surpasses” mark on the evaluation scale used. A list of professional goals for the next year was also set and approved. These goals either meet or exceed the goals set up within the scope of this training grant. The copy of a complete evaluation is included in the appendix 13.

In the year 2, the PI was also evaluated following the same process described above (in December-January 2012/2013). Based on this evaluation, PI has gotten an overall score of 4.4 out of 5 that corresponds to the “Surpasses” mark on the evaluation scale used. A list of professional goals for the next year was also set and approved. The copy of a complete evaluation is included in the appendix 14.

Table 2. Career Development Experiences and Timeline (training SOW).

Career Development Experiences / Timeline in Months	1-4	5-8	9-12	13-16	17-20	21-24
1. Structured mentoring program coordinated by the primary mentor through regularly scheduled weekly one-hour face-to-face meetings	x	x	x	x	x	x
2. Gain an understanding of the scientific paradigm used to develop, propose and implement bio-behavioral studies in minority populations	x	x				

3. Hands-on research experiences in scientific studies		X	X	X	X	X
4. Initiate and complete the research project	X	X	X	X	X	X
5. Begin developing bio-behavioral interventions and initiate her independent research plan for an independent position						X
6. Hands-on grant writing experiences			X	X	X	X
7. Research and educational meetings/journal club/grand rounds(1 per week)	X	X	X	X	X	X
8. Scientific writing and research dissemination			X	X	X	X
9. Interactions with established scientists, networking, and peer linkages	X	X	X	X	X	X

**Summary of the training program progress: all tasks up to date or completed as proposed in the training SOW.**

**B. Research Project.** Specific progress to date is outlined below.

12. **Specific Aim #1.** Evaluate different role(s) of genetic variations between AAM and EAM using the panel of select genetic markers. This will be achieved by an analysis of 600 germline DNA samples (300 AA and 300 EA), with each racial group containing 180 cases and 120 controls.
13. **Specific Aim #2.** Assess the genotype/phenotype correlations using the total of 120 AA samples (60 cases and 60 controls). This will be achieved by plasma analysis for expression of the most significant gene(s)/SNP(s), identified within the specific aim #1.
14. **Specific Aim #3.** To evaluate the feasibility of using the comprehensive DETECT approach in AAM as a PCa screening and risk assessment tool. Statistical measures to analyze the data obtained in the Aims #1-2, in addition to the medical charts and bio-behavioral questionnaire data, will be used. This will be achieved by: **3a.** Estimating the associations between the individual DETECT markers and PCa risk. **3b.** Establishing combined associations of elected (in sub-aim 2a.) markers and PCa. **3c.** Building and evaluating a prediction model of PCa risk based on results in Aim 2a and 2b.

**YEAR 1**

**Specific progress. Aim #1:** Evaluate different role(s) of genetic variations between AAM and EAM using the panel of select genetic markers. This will be achieved by an analysis of 600 germline DNA samples (300 AA and 300 EA), with each racial group containing 180 cases and 120 controls.

**SNP selection.** PI has performed extensive literature search, that allowed her to select 10 short nucleotide polymorphisms (SNPs) that were: 1. reported positively associated with prostate cancer (increased risk) in African American (desirable) and/or White men; 2. functional (i.e. possibly having an influence on the gene expression); 3. reported Minor allele frequency (MAF) at least 15% in both African / Black and European / White men. Based on these criteria, the following SNPs were selected (Table 3).

Table 3. Short description of the SNPs selected for the Aim #1 of this study. W: White men; AA: African American men; OR: odds ratio;

SNP	Functional (Y/N) and gene	notes	ref
<b>Rs4430796</b> G/A, Transition Substitution W:0.47(A) AA:0.34(A)	Possibly Y. TCF2 gene intronic region, possible effect on transcription	OR = 1.4	1
<b>Rs7501939</b> T/C, Transition Substitution W:0.49(T) AA:0.48(C)		OR = 1.44	

<b>Rs1859962</b> G/T, Transversion Substitution W:0.5(G) AA:0.2(G)		OR = 1.21  Associated with both overall, and familial/aggressive PCa in white men	
<b>Rs10993994</b> C/T, Transition Substitution W:0.34(T) AA:0.2(C)	Y; MSMB gene encodes PSP94; reduced promoter activity	OR = 1.3	2, 3
<b>rs822396</b> A/G, Transition Substitution W:0.15(G) AA:0.2(G)	Y; ADIPOQ	OR = 3	4
<b>Rs4263970</b> C/T, Transition Substitution W:0.45(C) AA:0.47(T)  <b>Rs4612601</b> A/G, Transition Substitution W:0.45(A) AA:0.5(G)	Y: EPHB2	OR= 1.31  OR = 1.35 Sporadic PCa in AAM only	5
<b>rs4962416</b> C/T, Transition Substitution W:0.23(C) AA:0.23(C)	Y: CTBP2 gene	OR = 1.17 – 1.63 in AAM	6
<b>Rs627839</b> G/T, transversion substitution W:0.45(T) AA:0.46(T)	Y: RNASEL	OR = 3.98 in White men (PCa specific death)	7
<b>Rs2070874</b> G/T, transition substitution W:0.2(T) AA:0.45(T)	Y: IL4	OR = 2.16 in White men (PCa specific death)	7

- Merging of the databases and creating the data dictionary. Because the samples came from two different and independent studies (Drs. Phelan and Park PIs), the PI had to create a merged data dictionary to be used in her study. This task was successfully accomplished. The data dictionary can be found in the appendix 15.
- The Institutional Review Board (IRB) and Scientific Review Committee (SRC) approvals were successfully obtained by the PI. The copies of both approvals are attached in the appendices 1 and 2, respectively.
- Sample selection. The PI has selected the samples for her study on the basis of matching. She has performed a pair wise extensive matching of cases and controls by race and ethnicity, age (+/- 2 years in most cases), Gleason score, TNM stage (cases), and PSA (where available).
- Genotyping was carried out by the PI in the Dr. Park's molecular biology laboratory. Applied Biosystems primer and PCR supplies were used to genotype the individual SNPs in all the samples.
- Data analysis was carried out using the SAS software.

**Results.** 528 germline DNA samples were successfully genotyped. That includes 259 samples obtained from African American men (136 cases and 123 controls), and 269 samples obtained from Non-Hispanic White men (147 cases and 142 controls). Each genotyping plate included 5-10% of duplicate samples, and concordance rates for all the genotyping experiments were 99% or more. Once the genotyping was completed, the dominant, recessive and additive age-adjusted models were fitted to explore the associations of individual SNPs with prostate cancer. We have presented the results for all men combined (cases vs controls), as well as African

American (AAM) and European American (EAM) men separately. Please see table 4 for detailed genotyping results. Significant associations are shown in **bold**.

Table 4. Age-adjusted genotyping models for prostate cancer.

SNP	Dominant Model OR (CI); p-value	Recessive Model OR(CI); p-value	Additive Model OR(CI); p-value
1. rs4612601	AAM: 1.09 (0.63-1.9); 0.749	0.86 (0.46-1.61); 0.638	0.99 (0.69-1.41); 0.951
	EAM: 1.18 (0.67-2.10); 0.564	0.9 (0.52-1.56); 0.709	1.02 (0.73-1.43); 0.912
	All: 1.13 (0.76-1.69); 0.537	0.89 (0.59-1.34); 0.565	1.00 (0.79-1.28); 0.969
2. rs4263970	AAM: 0.98 (0.57-1.68); 0.932	0.88 (0.44-1.75); 0.711	0.95 (0.66-1.38); 0.797
	EAM: 1.08 (0.61-1.9); 0.795	0.76 (0.44-1.33); 0.334	0.93 (0.66-1.30); 0.665
	All: 1.02 (0.69-1.5); 0.918	0.81 (0.53-1.25); 0.343	0.94 (0.73-1.21); 0.630
3. rs822396	AAM: 0.89 (0.52-1.52); 0.671	1.64 (0.37-7.21); 0.511	0.96 (0.6-1.54); 0.878
	EAM: 0.75 (0.44-1.27); 0.288	1.1 (0.29-4.09); 0.094	0.82 (0.53-1.29); 0.392
	All: 0.82 (0.56-1.19); 0.285	1.29 (0.49-3.48); 0.601	0.88 (0.64-1.22); 0.456
4. rs10993994	AAM: 0.82 (0.48-1.4); 0.472	1.04 (0.51-2.10); 0.919	0.92 (0.63-1.33); 0.657
	EAM: 1.04 (0.54-2.00); 0.899	0.61 (0.36-1.05); 0.077	0.81 (0.56-1.17); 0.258
	All: 0.9 (0.6-1.35); 0.611	0.75 (0.49-1.14); 0.177	0.87 (0.67-1.11); 0.261
5. rs1859962	AAM: 1.03 (0.61-1.74); 0.905	1.23 (0.54-2.81); 0.626	1.06 (0.72-1.56); 0.752
	EAM: 1.38 (0.77-2.45); 0.278	1.00 (0.57-1.76); 0.997	1.13 (0.79-1.6); 0.508
	All: 1.16 (0.8-1.69); 0.431	1.06 (0.67-1.69); 0.790	1.09 (0.85-1.4); 0.503
6. rs7501939	AAM: 1.34 (0.73-2.47); 0.343	<b>2.42 (1.31-4.47); 0.0046</b>	<b>1.56 (1.08-2.27); 0.0193</b>
	EAM: 0.82 (0.5-1.35); 0.439	1.13 (0.54-2.38); 0.742	0.93 (0.65-1.33); 0.692
	All: 0.99 (0.68-1.45); 0.981	<b>1.76 (1.11-2.79); 0.0167</b>	1.18 (0.92-1.52); 0.187
7. rs4430796	AAM: <b>0.57 (0.34-0.97); 0.0383</b>	0.64 (0.29-1.42); 0.272	<b>0.67 (0.46-0.99); 0.0431</b>
	EAM: 1.27 (0.7-2.3); 0.439	0.98 (0.57-1.68); 0.946	1.07 (0.76-1.51); 0.689
	All: 0.81 (0.55-1.18); 0.273	0.86 (0.59-1.33); 0.500	0.87 (0.69-1.12); 0.279
8. rs2070874	AAM: 0.94 (0.54-1.64); 0.824	1.45 (0.7-3.0); 0.314	1.08 (0.73-1.59); 0.700
	EAM: 0.65 (0.38-1.11); 0.115	0.92 (0.22-3.93); 0.916	0.72 (0.45-1.14); 0.161
	All: 0.82 (0.57-1.17); 0.275	1.32 (0.7-2.49); 0.386	0.94 (0.71-1.24); 0.649
9. rs627839	AAM: 1.2 (0.7-2.06); 0.511	1.63 (0.78-3.42); 0.192	1.24 (0.85-1.83); 0.252
	EAM: 0.86 (0.5-1.47); 0.571	0.99 (0.55-1.8); 0.986	0.94 (0.67-1.32); 0.710
	All: 1.01 (0.69-1.48); 0.952	1.21 (0.76-1.91); 0.427	1.06 (0.83-1.37); 0.632
10. rs4962416	AAM: 0.9 (0.5-1.59); 0.709	5.65 (0.62-51.7); 0.125	1.05 (0.63-1.74); 0.861
	<b>EAM: 1.69 (1.02-2.8); 0.0415</b>	1.72 (0.68-4.31); 0.251	<b>1.52 (1.02-2.26); 0.0384</b>
	All: 1.28 (0.88-1.85); 0.198	2.12 (0.92-4.9); 0.0784	1.31 (0.97-1.78); 0.083

The main findings from the Table 4 can be summarized as follows:

1. African American men carrying two copies of the rs7501939 SNP (homozygous for the C-allele) have more than twofold increase in prostate cancer risk (2.42 (1.31-4.47); 0.0046), and this increase in risk appears to be highly statistically significant. The minor allele frequency (MAF) in African American men is high (47.7% calculated in our study, 48% reported previously), and 24% of men are homozygous for the C-allele in our study. However, heterozygous men also appear to be at the statistically significantly increased risk (1.56 (1.08-2.27); 0.0193), albeit to a lesser extent. About 47 % of men were heterozygous in our study. Hence, the C-allele of the rs7501939 SNP appears to be a risk factor for prostate cancer in African American men.
2. African American men carrying two copies of the wild allele of rs4430796 SNP (homozygous for the G-allele) have decreased prostate cancer risk (0.57 (0.34-0.97); 0.0383). MAF (A-allele) in African American men is 31% calculated in our study, and 34% reported previously. However, heterozygous



men also appear to be at the statistically significantly reduced risk (0.67 (0.46-0.99); 0.0431). About 50% of men were homozygous of the wild allele, and 37 % of men were heterozygous in our study. Hence, the wild allele of the rs4430796 SNP (G-allele) appears to be protective of prostate cancer in African American men.

3. European American men carrying two copies of the wild allele of rs4962416 (homozygous for the T-allele) have increased prostate cancer risk (1.69 (1.02-2.8); 0.0415). MAF (C-allele) in White men is 29% calculated in our study, and 23% reported previously. However, heterozygous men also appear to be at the statistically significantly increased risk (1.52 (1.02-2.26); 0.0384). About 50% of men were homozygous of the wild allele, and 40 % of men were heterozygous in our study. Hence, the wild allele of the rs4962416 SNP (T-allele) appears to be a risk factor of prostate cancer in White men.
4. Since rs7501939 and rs4430796 are located in the same region of chromosome 17, we used the SNAP software to determine if they are in a linkage disequilibrium (LD). In White men, the SNPs were in a relatively strong linkage disequilibrium ( $r^2=0.76$ ); however, this did not appear to be the case in Black men ( $r^2=0.145$ ). Hence, we conclude that in African American men, the effects of those SNPs are independent of each other.
5. We have used the SNAP software to further look for SNPs that are in a strong ( $r^2 \geq 0.8$ ) and/or moderate LD ( $r^2 \geq 0.5$ ) with rs7501939 and rs4430796 in African American men, and rs4962416 in European American men. The results are shown in the Table 5. From these results, we conclude that the effects of our SNPs of interest, if indeed real, are coming from the SNPs itself and not from any other SNPs in the LD region.

Table 5. SNPs reported being in LD regions with our SNPs of interest.

SNP of interest	$r^2 \geq 0.8$	$r^2 \geq 0.5$	Disease and comments
rs7501939 in AAM	none	rs11657964 ( $r^2=0.61$ ) rs8064454 ( $r^2=0.549$ )	None reported being associated with prostate cancer or any other disease.
rs4430796 in AAM	none	none	NA
rs4962416 in EAM	rs4962720 ( $r^2=1.00$ ) rs4962419 ( $r^2=1.00$ ) rs12771627 ( $r^2=1.00$ ) rs12769019 ( $r^2=0.92$ ) rs11598549 ( $r^2=0.92$ ) rs12782469 ( $r^2=0.83$ )	14 additional SNPs	rs12769019: slightly increased risk for prostate cancer in EAM (OR=1.1) [8];

We have also started looking at the bio-behavioral risk factors that were previously reported being associated with prostate cancer. The results are shown in the Table 6. Additional variables will be analyzed in the year #2 of this award.

Table 6. Age-adjusted association between select anthropometric and behavioral variables and prostate cancer in men stratifying by race and combined. **Bold** denotes statistically significant associations; *italics* denotes statistically marginally significant associations.

	AAM	EAM	All
Height: OR (95% CI); p-value	0.98 (0.9-1.05); 0.544	<b>0.92 (0.85-0.99); 0.0434</b>	<i>0.95 (0.898-1.001); 0.0549</i>
BMI: OR (95% CI); p-value	<b>1.06 (1.008-1.114); 0.022</b>	0.98 (0.93-1.023); 0.332	1.02 (0.98-1.05); 0.374
Smoking: OR (95% CI); p-value	1.01 (0.99-1.03); 0.118	1.00 (0.99-1.01); 0.959	1.002 (0.99-1.01); 0.565

Since our results indicated that BMI might be positively associated with prostate cancer in African American men, we decided to look for possible associations between BMI and significant SNPs: rs7501939 and rs4430796. Although our numbers were small, the results are shown in Table 7.

Table 7. rs7501939 and rs4430796 as risk factors for prostate cancer in African American men stratified by the body weight/BMI. Statistically significant associations are shown in **bold**.

	Non-obese AAM: BMI<30; 55 cases and 69 controls	Obese AAM: BMI≥30; 79 cases and 49 controls	All AAM (data from table 4)
rs7501939	1.09 (0.64-1.83); 0.763	<b>2.14 (1.2-3.8); 0.0103</b>	<b>2.42 (1.31-4.47); 0.0046</b>
rs4430796	0.84 (0.49-1.43); 0.513	0.67 (0.37-1.2); 0.181	<b>0.57 (0.34-0.97); 0.0383</b>

Similarly, we have also tested the association of rs7501939 and prostate cancer in White men by obesity status. No statistically significant association was seen in either non-obese (p=0.3) or obese (p=0.8) men of European descent.

As could be seen from data shown in Table 7, we were either underpowered to detect the effect of obesity in the rs4430796 carriers, or there's no apparent association. Interestingly, the ORs (point estimates) were still in the protective range (less than 1.0) in both groups; however that observation did not attain statistical significance perhaps due to decreased sample size in both groups.

Interestingly, the rs7501939 remained a significant risk factor for prostate cancer in obese AAM even considering a small sample size, and the excessive risk was still more than twice. However, rs7501939 no longer appeared to be a risk factor for prostate cancer in the non-obese AAM.

Hypothesis to be tested in the specific aim #2: in obese (diabetic?) AAM, there may be active obesity-related mechanisms that alone do not lead to increased PCa risk; however, the presence of the rs7501939 C-allele may either cause additional risk-increasing mechanisms, or enhance the ones related to obesity, leading to more than twofold increased PCa risk. In the non-obese (non-diabetic?) men, the rs7501939 C-allele alone may not be sufficient to increase the PCa risk. Please see schematic illustration of this hypothesis (Figure 1).

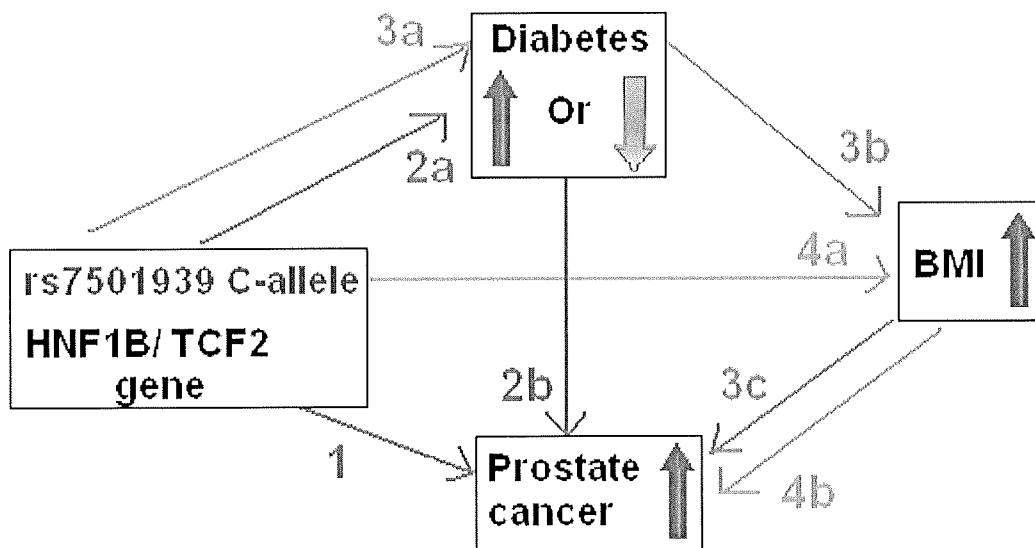


Figure 1. Possible pathways involved in the increased prostate cancer risk in rs7501939 C-allele African American carriers. 1 (blue line): direct association; 2a and 2b (magenta lines): association mediated through increased or decreased diabetes risk; 3a, 3b and 3c (grey lines): association mediated through increased or decreased diabetes risk and associated BMI increase; 4a and 4b (green line): association mediated through increased BMI.

## YEAR 2

**Specific progress. Specific Aim #2.** *Assess the genotype/phenotype correlations using the total of 120 AA samples (60 cases and 60 controls). This will be achieved by plasma analysis for expression of the most significant gene(s)/SNP(s), identified within the specific aim #1.*

**Rationale.** The need for an effective diagnostic and/or screening test, with higher sensitivity and specificity towards malignant prostate tumors in African American men, has been recognized [8]. This need drives the search for diagnostic biomarkers, specific to the high risk population of African Americans. Among the non-specific markers, OPG [9] and p53 [10] are recognized as indirect markers for prostate cancer progression and prognosis, however, their utility as serum detection and screening biomarkers was not explored in a high risk cohort of African American men. In the present study, we hypothesized that accuracy of the Serum Prostate Specific Antigen (PSA) prostate cancer screening test can be improved by serum p53 and OPG, which are established non-specific biomarkers for prostate cancer progression and prognosis.

**Methods.** The study is approved by the University of South Florida Institutional Review Board (IRB#104213).

Cases are African American men with biopsy-proven prostate cancer. Controls are African American men with low PSA and no other evidence of biopsy by prostate cancer. The cases and controls have come from screening at Lifetime Cancer Screening and prevention Center at the H. Lee Moffitt Cancer Center and Research Institute, the Moffitt Cancer Center Hospital, Tampa Bay Radiation Oncology centers, and 30<sup>th</sup> Street Medical Associates.

Written informed consent is obtained from each of the men in this study in accordance with local institutional review board approval. Human subjects in this study are asked to complete a questionnaire about general health risk factors, and family history, and provide blood samples.

Data were obtained from medical records by study personnel. Operative and pathology reports are obtained by study personnel from the office of the diagnosing physician. From these reports, we verified prostate cancer stage, grade, histological type, size of tumor and extent of surgical treatment. Data abstracted from these reports was reviewed in conjunction with review of pathology slides from the surgical specimen by a single pathologist, who verified disease grade and histological type. The results from the operative and pathology results determined whether a study volunteer is classified as 'case' if cancer is present or 'control' if cancer is not present.

Two vials of blood will be collected from each subject, Ten ml of blood was drawn (by the phlebotomist or nurse) into each tube prior to intervention or treatment (which may affect the serum and plasma proteins). Both tubes will be brought to Tissue Procurement at the Moffitt Cancer Center.

The 10ml purple top EDTA tubes will be centrifuged within 24 to 72 hours of blood drawing at 2500g for 15 minutes for plasma separation and buffy coat extraction for DNA. Plasma will be transferred and stored in microtubes at -70 °C. The buffy coat in the EDTA-tube will be placed in a 50ml falcon tube, and RBC lysis buffer added and the tube incubated for 30 minutes. The tube is then vortexed, and cell lysis buffer added and the tube incubated at room temperature overnight. Then the sample is treated to remove RNA and proteins, prior to and isopropanol ethanol wash and the DNA is resuspended in Tris-EDTA buffer, as per manufacturers' instructions (Puregene, Gentra systems, MN, USA). The DNA is stored at 4°C. The red tiger topped tube will be spun down as soon as possible to remove the sera. The sera will also be stored at -70 °C. Sera, Plasma and DNA samples will be stored indefinitely for this and other studies as long as the integrity of the sample is maintained.

Commercially available ELISA kits were purchased from Abnova (product numbers: PSA - KA0208; OPG – KA0030; p53 – KA0155) and were stored and used according to manufacturer's instructions. Phosphate buffered saline (PBS) tablets were purchased from Sigma Aldrich. PBS solution (10 mM, pH 7.4) was prepared by dissolving 1 tablet in 200 ml of deionized water. Synergy HT microplate reader was purchased from Biotek.

**Statistical analyses.** Frequency and percentages of the discrete variables were computed. Pearson's Chi-square test and Fisher's exact test were used to test the independence between discrete variables and cancer status as being positive (case) or negative (control). Wilcoxon rank-sum test was used to compare the distributions of each continuous variable between the case and control groups. Univariable and multivariable logistic regression

models were built to test the marginal and joint effects of the variables when predicting the cancer status. Point and interval estimates of the odds ratio as well as the p-value of each of the variables were reported. All p-values less than 0.05 were considered significant. Statistical analyses were conducted using Statistical Analysis System (SAS) software, version 9.2 (SAS Institute, Cary, NC).

Results. Total of 29 cases and 32 controls were tested. Age and stage characteristics are shown in Table 8. Despite the best effort to perform a close age matching, cases were, on average, older than controls; however, an average age of cases in our cohort was still 7 years younger than an average nationwide age at the time of PCa diagnosis (69 years). This reflects the general trend that African American males are on average 3-5 years younger than men of other racial groups at the time of initial prostate cancer diagnosis [11]. All the cases in our study carried a moderately differentiated PCa at time of diagnosis. There were no patients with advanced or very initial stages of PCa. One case and four controls were below the recommended starting age for PCa screenings (50 years [12]) which also reflects the nationwide trend observed in African American men.

Table 8. Patients' characteristics.

PCa status/characteristic	Age range (average), years	Gleason score range	T stage range
Cases (+)	44 – 74 (62)	6-8	T1 – T2
Controls (-)	38 – 73 (56.5)	N/A	N/A

As expected, all the samples tested, including the cases and controls, expressed PSA. Average PSA for cases is 6.1 ng/ml, and 1.3 ng/ml for controls. In addition, all samples tested had detectable OPG expression. The average expression for cases was 328 pg/ml, and 289 pg/ml for controls. However, only 25% of samples had detectable p53 expression, including 8 cases and 7 controls. The average expression for cases was 6.12 u/ml, and 25.99 u/ml for controls. The summary of each biomarker expression is shown in Table 9.

Table 9. Expression of biomarkers

	PSA	P53	OPG
Total tested	<b>61</b>	<b>60</b>	<b>60</b>
Cases (+)	29	28	28
Controls (-)	32	32	32
<b>Expression detected (ED) total</b>	<b>61 (100%)</b>	<b>15 (25%)</b>	<b>60 (100%)</b>
ED cases	29 (100%)	8 (29%)	28 (100%)
ED controls	32 (100%)	7 (22%)	32 (100%)
<b>Average cases</b>	<b>6.1 ng/ml</b>	<b>6.12 u/ml</b>	<b>328 pg/ml</b>
<b>Average controls</b>	<b>1.3 ng/ml</b>	<b>25.99 u/ml</b>	<b>289 pg/ml</b>

As expected, PSA expression was significantly correlated with PCa status: OR=2.85 (95%CI: 1.72-4.73). The strong association is clearly illustrated by the ROC curve (Figure 2).

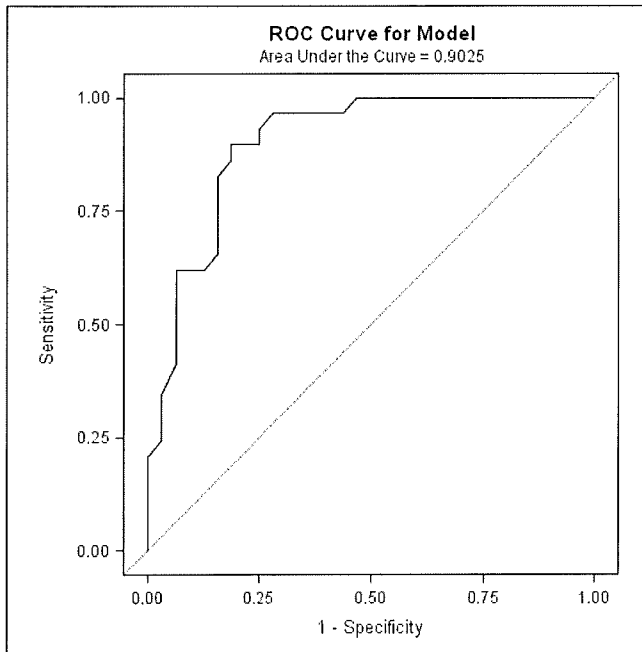


Figure 2. ROC curve for PSA

In contrast, neither OPG [OR=1.003 (95%CI: 0.998~1.007)], nor p53 [OR=0.936 (95%: 0.79-1.11)] expression was statistically significantly correlated with PCa status. Figure 3 depicts the absence of correlation by illustrating the ROC curve for OPG. Due to the limited number of samples with detectable p53 expression, no ROC was generated.

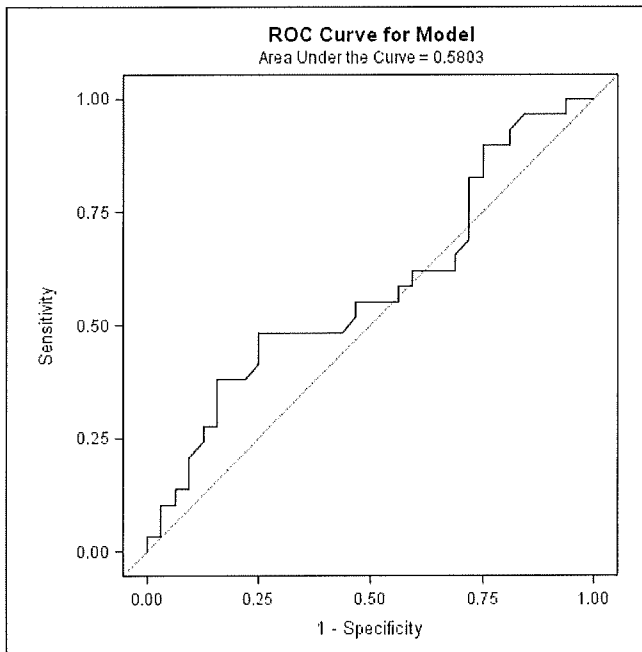


Figure 3. ROC curve for OPG.

In addition, no significant association was found between the 3 biomarkers which are illustrated in Table 10.

Table 10. Pearson coefficient (p value) for the three biomarkers studied

	PSA	OPG	P53
PSA	N/A	0.15 (0.25)	0.19 (0.46)
OPG		N/A	0.11 (0.67)
P53			N/A

Discussion. In this work, we thought to evaluate the utility of serum OPG and p53 as independent or joint with serum PSA screening or diagnostic biomarkers of prostate cancer in African American men. The rationale behind our work was that, in addition to higher prostate cancer incidence and mortality in African American population, there is no reliable screening or early detection tools that will allow sensitive and specific detection of prostate cancer. The rationale behind selecting OPG and p53 was that, increased serum OPG and p53 levels are also associated with increased tumor stage, tumor grade and early recurrence in myelomas, lung, bladder, breast, colorectal and, most importantly, prostate cancers [13]. In prostate cancer, increased OPG serum levels were linked to the disease progression [9], and thus OPG was declared to be a promising potential candidate for prostate cancer detection and progression in a pilot experiment with comparable sample sizes [14]. It was proposed that OPG may protect the tumor cells from apoptosis [15]. The need for additional experiments to further investigate the role of serum OPG in prostate cancer was therefore warranted.

P53 is an important tumor suppressor gene and its product – p53 protein - is often either down-regulated or mutated and up-regulated during malignant transformation and progression [10]. Although well studied, the role of p53 in cancer is elusive: the wild-type p53 may possess pro-apoptotic properties and be down regulated in the process of tumorigenesis [16], while mutated p53 may be up regulated because it suppress apoptosis and induces proliferation [17]. We aimed to explore, if any of these scenarios can be detected in serum of African American patients with moderately differentiated prostate cancer, and if this data can be used to improve the diagnostic or prognostic tools for this type of malignancy. It is notable, however, that no information on the levels of either OPG or p53 in circulation of African American males with any type of cancer, including carcinoma of the prostate, exists to date, which makes our work highly innovative with a potential to yield novel, important results which may not only drive the field of prostate cancer diagnostics forward, but also contribute to the resolution of cancer related disparities.

Based on the results presented earlier, the main outcome of our study can be formulated as such: no significant association between OPG or p53 and prostate cancer was found in our pilot cohorts of African American men with moderately differentiated prostate cancer, neither the levels of OPG and/or p53 were found useful to improve the detection value of PSA. This finding is in concordance with [14], who found significant association only between the serum OPG levels and advanced, untreated PCa. No difference in OPG levels was found in sera from patients with low to moderate grade prostate cancer, and healthy males of similar ages. The authors concluded that serum OPG may hold promise as a marker of progression and metastatic spread, but not the marker of early detection or screening.

### **Future directions**

During the approved 1-year no cost extension of this project, we plan to investigate the *HNF1B* expression in plasma, and/or prostate cancer cell lines, and/or prostate cancer tumor and normal tissues obtained from AAM and EAM. The experiments will be conducted using the same or similar methodology, patients and study design described above. The selection of the *HNF1B* gene was based on our data described in the Aim 1 of this study. The rationale behind this work is to connect the germline variation in the *HNF1B* and obesity to prostate cancer risk through the functional studies.

**Specific progress. Specific Aim #3.** *To evaluate the feasibility of using the comprehensive DETECT approach in AAM as a PCa screening and risk assessment tool. Statistical measures to analyze the data obtained in the Aims #1-2, in addition to the medical charts and bio-behavioral questionnaire data, will be used. This will be achieved by: 3a. Estimating the associations between the individual DETECT markers and PCa risk. 3b. Establishing combined associations of elected (in sub-aim 2a.) markers and PCa. 3c. Building and evaluating a prediction model of PCa risk based on results in Aim 2a and 2b.*

Rationale. The trinucleotide (CAG)(n) repeat polymorphism in exon 1 of the androgen receptor (AR) gene regulates AR activity, with longer alleles conferring reduced activity (Figure 4 [18]). Significant interethnic variations in the allele length frequencies of the AR (CAG)(n) polymorphism repeat have been reported [19]. We investigated the association between the AR CAG(n) repeat length and prostate cancer risk in AAM and EAM. We further explored if this association varied by body mass index (BMI) in the two races.

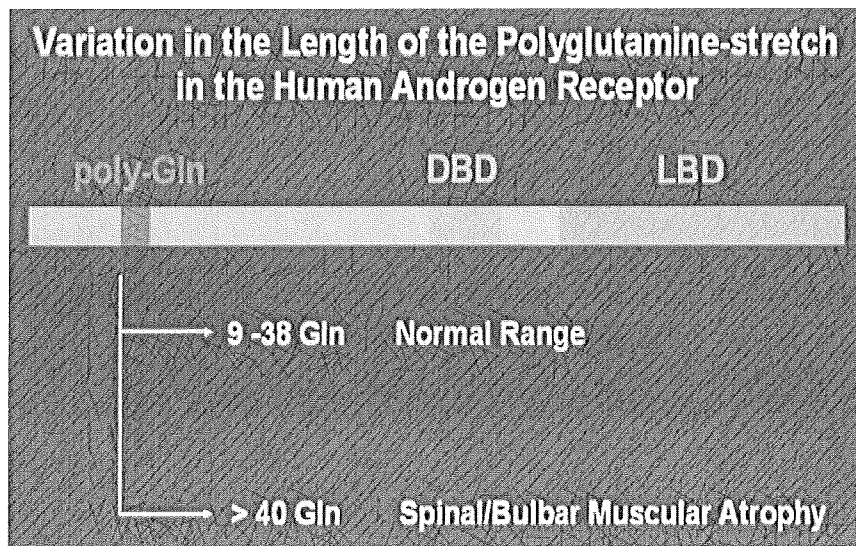


Figure 4. Schematic of the human androgen receptor. DBD = DNA binding domain; LBD = ligand binding domain; poly-GLN = polyglutamine repeats (CAG) [18].

**Methods.** Study participants were recruited from various hospitals in Tampa Bay. Eligible subjects self-classified as either AAM or EAM and were 30-80 years old. Cases had biopsy-confirmed prostate cancer and had no prior treatment at the time of enrollment. Controls were defined as men having no evidence of prostate cancer on biopsy and/or low PSA levels. All men signed an informed consent form and provided a blood or saliva sample for DNA. We tested DNA samples from 259 (136 cases and 123 controls) AA and 269 (147 cases and 142 controls) NHW men, matched by age, Gleason score and PSA. The CAG repeat region was PCR amplified, and the PCR fragments were analyzed using the Beckman Coulter CEQ8000 DNA sequencer to establish the repeat length.

**Statistical analyses.** Descriptive statistics was performed for discrete and continuous variables. Associations between repeats and prostate cancer risk were evaluated using unconditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (95%CI) for each race. Repeats were categorized using the median (22 repeats) as a cutpoint. Significance was defined as the p-value <0.05.

**Results.** DNA samples from 259 (136 cases and 123 controls) AAM and 269 (147 cases and 142 controls) EAM were analyzed, yielding a total sample size of 528 men. EAM were matched to AAM by case status, age, and Gleason score. Select characteristics of the study participants by case/control status are shown in the Table 11. For both ethnic groups, men were likely to be between 50 to 64 years of age and between 68 and 72 inches tall. Within ethnic groups, AAM cases were more likely to be obese than AAM controls. In contrast, EAM controls were more likely to be obese than EAM cases. AAM were more likely to be obese and taller than EAM. However, these differences were not statistically significant (p>0.05).

Table 11. Select characteristics of the study participants by case/control status.

		<b>AAM: 259 total</b>		<b>EAM: 269 total</b>	
		# Cases (%)	# Controls (%)	# Cases (%)	# Controls (%)
		136 (52.5)	123 (47.5)	147 (54.6)	122 (45.4)
<b>Age</b>	<50	11 (8)	30 (24)	12 (8)	19 (16)
	50-64	74 (54)	73 (59)	80 (54)	85 (70)
	≥65	51 (38)	17 (14)	55 (38)	18 (14)
	missing	NA	3 (3)	NA	NA
<b>BMI</b>	≤24.9 normal weight	9 (7)	26 (21)	25 (17)	28 (23)
	25.0-29.9	47 (34.5)	46 (37)	70 (48)	47 (38)

	overweight				
	≥30.0	71 (52)	51 (42)	48 (33)	46 (38)
	obese				
	missing	9 (6.5)	NA	4 (2)	1 (1)
<b>Height</b>	≤67 in	30 (22)	21 (17)	26 (17.5)	22 (18)
	68-72 in	65 (48)	74 (60)	98 (66.5)	79 (65)
	≥73 in	32 (23.5)	28 (23)	19 (13)	21 (17)
	missing	9 (6.5)	NA	4 (3)	NA

Results for the CAG repeat analysis and prostate cancer status are shown in the Table 12. No statistically significant associations were noted when the repeat length was treated as a continuous variable. However, when a binary cutpoint was used (a median repeat length in our study, CAG=22), AAM with longer repeats (≥22) demonstrated increased prostate cancer risk. Interestingly, the increased risk was only evident in obese (BMI≥30), but not in the non-obese (BMI<30) AAM. No associations were noted in EAM regardless of the BMI.

We have analyzed the CAG repeat distribution using other categories commonly used in other studies (<19; 12-25; and ≥26 repeats). None of the analyses were significant using these categories (p>0.05).

Table 12. AR CAG-repeat length and prostate cancer status. “+” denotes a case; “-” denotes a control.

	AAM				EAM				Combined			
	+	-	OR (95%CI)	P	+	-	OR (95%CI)	P	+	-	OR (95%CI)	P
Continuous	147	122	1.00 (0.94-1.07)	0.94	136	123	1.08 (1.00-1.17)	0.06	283	245	1.04 (0.99-1.09)	0.17
Binary all												
<22	40	49	Reference		66	66	Reference		106	115	Reference	
≥22	86	63	1.81 (1.05-3.13)	0.034	53	50	0.96 (0.56-1.65)	0.87	136	116	1.37 (0.90-1.92)	0.15
Non-obese												
<22	30	31	Reference		28	38	Reference		58	69	Reference	
≥22	54	39	1.43 (0.73-2.77)	0.30	19	33	0.80 (0.35-1.83)	0.60	73	72	1.22 (0.73-2.01)	0.45
Obese												
<22	9	18	Reference		34	28	Reference		43	46	Reference	
≥22	29	23	3.12 (1.12-8.73)	0.03	27	20	1.11 (0.51-2.41)	0.79	56	43	1.45 (0.18-2.60)	0.21

**Discussion.** Obesity (BMI≥30) alters individual’s hormonal profile; specifically, estrogens are increased, and androgens are decreased in obese compared to normal weight men [20]. While the majority of early prostate cancers rely on androgens, depletion of androgens (castration) eventually leads to an androgen independent malignancy [21]. Our provocative, very preliminary findings may indicate that obese AAM with altered androgen/estrogen profile (androgen ↓, estrogen ↑) and reduced activity AR receptors may be more susceptible to develop prostate cancers (Table 13). Further, we are tempted to hypothesize that obese AAM with longer CAG repeats are prone to develop an androgen-independent malignancy that may be more aggressive and/or resistant to conventional treatments. Additional research is needed to test this hypothesis as well as establish possible reasons why this effect is observed in AAM but not in EAM.



Table 13. Androgen receptor (AR) activity, androgen/estrogen balances and prostate cancer in AAM

AR activity	Androgen/estrogen	
	Normal/normal (BMI<30)	Low/High (BMI≥30)
Normal (CAG<22)	Conventional slow-growing prostate cancer	? Still conventional slow-growing cancer ?
Reduced (CAG≥22)	? Still conventional slow-growing prostate cancer ?	<b>Androgen-independent, aggressive cancer</b>

Conclusions. Our results suggest that prostate cancer risk may be increased in AAM with longer AR CAG repeats (≥22), especially obese (BMI≥30) AAM. The data provide evidence for ethnic differences in androgenic pathway activity and androgen sensitivity which may contribute to prostate cancer disparity.

#### **Future directions**

During the approved 1-year no cost extension of this project, we plan to continue the data analysis and secure the follow up funding to continue this work with the intention to advance it on the level of functional tumor biology.

**Summary of the research progress: Aim #1: completed. Aims 2 and 3 – partially completed, pending completion during the approved no-cost extension.**

#### **Key Research Accomplishments**

##### Year 1:

1. 528 germline DNA samples were successfully genotyped for selected functional SNPs. That includes 259 samples obtained from African American men (136 cases and 123 controls), and 269 samples obtained from Non-Hispanic White men (147 cases and 142 controls).
2. Based on the genotyping results, three SNPs showed statistically significant associations with prostate cancer. In African American men: rs7501939 C-allele (OR 2.42; CI 1.31-4.47; p=0.0046) and rs4430796 G-allele (OR 0.57; CI 0.34-0.97; p=0.0383). In European American men: rs4962416 T-allele (OR 1.52; CI 1.02-2.26; p=0.0384).
3. Select anthropometric variables were statistically significantly associated with prostate cancer in our cohort of men. In African American men: BMI (OR 1.06; CI 1.008-1.114; p=0.022). In European American men: height (OR 0.92; CI 0.85-0.99; p=0.0434).
4. Interestingly, rs7501939 C-allele appeared to be a risk factor for prostate cancer in obese (BMI ≥30) African American men only (OR 2.14; CI 1.2-3.8; p=0.0103). It was no longer a risk factor in the non-obese African American men.
5. Neither rs4430796 nor rs4962416 showed statistically significant associations with BMI and/or height.

##### Year 2:

6. No significant association between OPG or p53 and prostate cancer was found in our pilot cohorts of African American men with moderately differentiated prostate cancer, neither the levels of OPG and/or p53 were found useful to improve the detection value of PSA.
7. No difference in OPG levels was found in sera from patients with low to moderate grade prostate cancer, and healthy males of similar ages.
8. 528 germline DNA samples were successfully genotyped for the CAG repeat length in the androgen receptor gene. That includes 259 samples obtained from African American men (136 cases and 123 controls), and 269 samples obtained from Non-Hispanic White men (147 cases and 142 controls).
9. Based on the genotyping results, no statistically significant associations were noted when the repeat length was treated as a continuous variable. However, when a binary cutpoint was used (a median repeat

length in our study, CAG=22), AAM with longer repeats ( $\geq 22$ ) demonstrated increased prostate cancer risk.

10. Interestingly, the increased risk was only evident in obese ( $\text{BMI} \geq 30$ ), but not in the non-obese ( $\text{BMI} < 30$ ) AAM. No associations were noted in EAM regardless of the BMI.
11. We have analyzed the CAG repeat distribution using other categories commonly used in other studies ( $< 19$ ; 12-25; and  $\geq 26$  repeats). None of the analyses were significant using these categories ( $p > 0.05$ ).
12. On the basis of our data, we are tempted to hypothesize that obese AAM with longer CAG repeats are prone to develop an androgen-independent malignancy that may be more aggressive and/or resistant to conventional treatments. Additional research is needed to test this hypothesis as well as establish possible reasons why this effect is observed in AAM but not in EAM.

### **Reportable Outcomes**

#### **1. Grants submitted:**

##### 1<sup>st</sup> year:

- NIH R03 1R03CA172753-01: Comparative race-specific chemopreventive effects of curcumin in prostate cancer; submitted on 02/2012; [not funded]
- DoD hypothesis development PC120156: Comparative race-specific chemopreventive effects of curcumin in prostate cancer; submitted on 06/2012. [not funded]

##### 2<sup>nd</sup> year:

- NIH R21 1R21CA179659-01: Obesity and prostate cancer risk in Black and White men: a functional approach; submitted on 11/2012; scored 3.9 (30<sup>th</sup> percentile).
- NIH R21 1R21CA179659-01-Resubmission: Obesity and prostate cancer risk in Black and White men: a functional approach; will be submitted 07/2013.

#### **2. Posters presented:**

##### 1<sup>st</sup> year:

- Joining FORCES Against Hereditary Cancer Conference (June 2011, Orlando FL). The poster presentation entitled "Risk of Gastrointestinal Cancers in Female BRCA1 and BRCA2 Mutation Carriers".
- Center for Equal Health Strategic Planning Retreat Meeting (September 2011).
- Annual AACR meeting (March-April 2012, Chicago IL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".
- The First Florida Prostate Cancer Research Symposium (May 4-5 2012, Orlando FL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".
- Moffitt's 2012 Scientific Symposium (May 10 2012, Tampa FL). The poster presentation entitled "The Risk Factors for Prostate Cancer in African American Men".

##### 2<sup>nd</sup> year:

- The Second Florida Prostate Cancer Research Symposium (March 2013, Orlando FL). The poster presentation entitled "Androgen Receptor CAG Repeat Length and Association with Prostate Cancer Risk in Obese and Non-Obese African American men".

- AACR Annual Meeting (04, 2013): Variation in transmembrane transport genes influence epithelial ovarian cancer risk and histopathologic subtype (poster presentation). Authors: Chornokur G; Tyrer J; Lin H-Y; Han G; Qu X; Chen Z; Tsai Y-Y; Goode EL; Cunningham JM; Iversen E; Ramus S; Berchuck A; Schildkraut JM; Monteiro A; Gayther S; Narod SA; Pharoah P; Sellers TA; Phelan CM.

- NIH “2012 Science of Eliminating Health Disparities Summit” (12, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation). Authors: Chornokur G; Amankwah EK; Park JY; Phelan CM; Kumar NB.

- AACR “The Science of Health Disparities” Conference (10, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation). Authors: Chornokur G; Amankwah EK; Park JY; Phelan CM; Kumar NB.

The PI has received a competitive Scholar-in-training Award to cover the travel/attendance cost for this meeting.

### 3. Manuscripts (published, accepted for publication or submitted):

#### 1<sup>st</sup> year:

- Shahnjayla Connors, **Ganna Chornokur**, Nagi Kumar. New Insights to the Mechanisms of Green Tea Catechins in the Chemoprevention of Prostate Cancer. Nutrition and cancer. 11/2011; 64(1):4-22; PMID: 22098273

- Kumar NB, Crocker, T, Smith T, Pow-Sang, J, Spiess, PE, Connors, SK, **Chornokur, G**, Iravani Dickinson, S, Williams, CR, Salup, R, Bai, W. Prostate Cancer Chemoprevention targeting High Risk Populations: Model for trial design and outcome measures. J Cancer Sci Ther. 2012; PMID: 22422102

- T.G. Kryshchak L.V. Borkovska O. Kolomys N.O. Korsunskaya V.V. Strelchuk, L.P. Germash, K.Yu. Pechers'ka, **G. Chornokur**, S.S. Ostapenko, C.M. Phelan, O.L. Stroyuk. The effect of bio-conjugation on aging of the photoluminescence in CdSeTe-ZnS core-shell quantum dots. Superlattices and Microstructures 51(3) 2012, 353–62

- Manju Venugopal, Sunil K. Arya, **Ganna Chornokur**, Shekhar Bhansali. A Realtime and Continuous Assessment of Cortisol in ISF Using Electrochemical Impedance Spectroscopy // Sens Actuators A Phys. 2011 1;172(1):154-160. PMID: 22163154

#### 2<sup>nd</sup> year:

- **Chornokur G.**, Kumar NB. Prostate cancer chemoprevention in men of African descent: current state of the art and opportunities for future research. Cancer Causes and Control 2013 [in press]. DOI: 10.1007/s10552-013-0241-x. PMID: 23737026

- Kumar N, **Chornokur G** (2012) Molecular Targeted Therapies Using Botanicals for Prostate Cancer Chemoprevention. Transl Med S2: 005. doi:10.4172/2161-1025.S2-005

- **Chornokur G**; Han G; Tanner R; Lin H-Y; Green LB, Pow-Sang J; Phelan CM. High Grade Prostate Intraepithelial Neoplasia (PIN) is a PSA-independent risk factor for prostate cancer in African American men: results from a pilot study. Cancer Letters 331(2): 154-7, 2013. PMID: 23268329

- **Chornokur G**, Amankwah EK, Schildkraut JM, Phelan CM. Global ovarian cancer health disparities. Gyn Oncol 121(1): 358-64, 2013. PMID: 23266352

- **Chornokur G**, Amankwah EK, Davis SN, Phelan CM, Park JY, Pow-Sang J, Kumar NB. Variation in *HNF1B* and obesity may influence prostate cancer risk in African American men: a pilot study. Submitted to "Cancer Prevention Research", 06/2013.

- Phelan CM, Iqbal J, Lubinski J, Ragone A, Cybulski C, Lynch HT, Moller P, Ghadirian P, Foulkes WD, Armel S, Neuhausen SL, Senter L, Singer CF, Kim-Sing C, Tung N, Llacuachaqui M, **Chornokur G**, Ping S, Narod SA and the Hereditary Breast Cancer Study Group. Incidence of colorectal cancer in *BRCA1* and *BRCA2* mutation carriers: Results from a follow-up study. Submitted to Br. J. Cancer, 05/2013.

- **Chornokur G**, Amankwah EK, Lin HY .... and Phelan CM on behalf of OCAC. Cellular Transport Gene Variants Are Associated With Susceptibility to Epithelial Ovarian Cancer Risk and Histopathologic Subtypes. In preparation. Targeted timeline for submission 08/2013.

#### **4. Classes / courses taken or audited:**

##### 1<sup>st</sup> year:

- Cancer Epidemiology (Moffitt Cancer Center and University of South Florida);
- Biostatistics 101 (Moffitt);
- NIH grant writing workshop (AACR Annual Meeting);
- Human Subjects Protection refresher course (Moffitt Cancer Center and University of South Florida);
- Biosafety refresher course (Moffitt Cancer Center and University of South Florida);
- Moffitt annual Mandatory education test (Moffitt);

##### 2<sup>nd</sup> year:

- Cancer Genetics (Moffitt);
- Moffitt annual Mandatory education test (Moffitt);

#### **5. Awards received by the PI:**

##### 2<sup>nd</sup> year:

Scholar-in-training Award to attend the Fifth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, 10, 2012

### **Conclusions**

Both the training program and the research supplemental project are up to date, as described in the SOW in the original proposal.

On the training part, the PI advanced her knowledge and expertise in prostate cancer health disparity. The main training activities include, but are not limited to, regular meetings with the Mentoring team; gain hands-on research experience in the implementation and conduct of research studies; writing and submitting grants and manuscripts; attending relevant high-impact scientific meetings and conferences; making presentations; taking relevant courses and classes; and attending local seminars, ground rounds, and/or workshops focused on the topics of cancer (including prostate cancer), health disparities, community outreach and molecular biology/genetics.

On the research part, in the year 1, the PI has successfully genotyped 528 germline DNA samples obtained from AAM and EAM with prostate cancer and controls. For the genotyping experiments, she has selected 10 functional SNPs previously reported being associated with increased prostate cancer risk. It was found that two SNPs were statistically significantly associated with prostate cancer risk in AA, and one SNP – in EA men. Going further, the excessive prostate cancer risk associated with one of the SNPs in AA men was found in obese men only; it was not seen in either non-obese AAM, or EAM of any body mass.

In the year 2, the PI has proposed a concept of the increased risk in obese AAM based on the results of her year 1 results. Simultaneously, the PI has genotyped the same 528 AA/EA germline samples for CAG repeat in the androgen receptor gene. Data analysis revealed that no statistically significant associations were noted when the repeat length was treated as a continuous variable. However, when a binary cutpoint was used (a median repeat length in our study, CAG=22), AAM with longer repeats ( $\geq 22$ ) demonstrated increased prostate cancer risk. Interestingly, the increased risk was only evident in obese (BMI $\geq 30$ ), but not in the non-obese (BMI $< 30$ ) AAM. No associations were noted in EAM regardless of the BMI. On the basis of these data, we are tempted to hypothesize that obese AAM with longer CAG repeats are prone to develop an androgen-independent malignancy that may be more aggressive and/or resistant to conventional treatments. Additional research is needed to test this hypothesis as well as establish possible reasons why this effect is observed in AAM but not in EAM.

In summary, during the duration of this award, the PI has submitted 4 peer-reviewed grants (including one resubmission); presented her work at 8 relevant national meetings and conferences; published 8 peer-reviewed manuscripts (including 3 first-author) with additional 3 manuscripts being under review or in preparation; has taken/audited 8 relevant classes and/or workshops; and received 1 competitive travel award. Most importantly, the PI has developed an intriguing hypothesis that she intends to obtain peer-review funds and pursue in her career.

A “so what section”: prostate cancer health disparity has a recognized genetic basis. It may, however, be challenging to translate the genetic data from bench to bedside due to the inherent complexity of the gene-gene, as well as gene-environment interactions. In this study, we have identified the functional SNPs that are significantly associated with prostate cancer risk in Black and White men. Further, we determined that the effect of these SNPs, as well as the length of CAG repeat polymorphism, on prostate cancer risk appears to correlate with obesity status and race. Our preliminary, hypothesis generating data has important public health implications for the US population, as 35-40% of people are obese and 1 in 5 AAM will be diagnosed with prostate cancer. Dr. Chornokur and her research team have developed a systematic project to attempt to tease out the biological basis for the observed associations between obesity, prostate cancer, race and androgen metabolism. While our data is preliminary, we strongly believe that our comprehensive approach will allow to shed light on the important question of the individual’s genetic variance, obesity and its connection to prostate cancer risk in the susceptible population of AAM.

## References

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## Appendices

1. IRB approval
2. SRC approval
3. Specific aims of the submitted R03
4. Specific aims and summary statement of the R21
5. *BRCA* cancers poster
6. Prostate AAM poster
7. *HNF1B* poster
8. Ovarian transport poster
9. AR CAG repeats poster
10. *HNF1B* manuscript (submitted to Cancer Prevention Research)
11. *BRCA* manuscript (submitted to British Journal of Cancer)
12. Ovarian transport paper (in preparation)
13. Moffitt postdoctoral evaluation 2011-2012
14. Moffitt postdoctoral evaluation 2012-2013
15. Merged data dictionary
16. No cost extension approval (06/2013-05/2014)

App. 1



UNIVERSITY OF  
SOUTH FLORIDA

DIVISION OF RESEARCH INTEGRITY AND COMPLIANCE

Institutional Review Boards, FWA No. 00001669  
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799  
(813) 974-5638 • FAX (813) 974-5618

7/21/2011

Ganna Chornokur  
H Lee Moffitt Cancer Center  
12902 Magnolia Dr.

RE: **Exempt Certification** for IRB#: Pro00004885

Title: Comprehensive Population-Specific Marker Panel for Early Prostate Cancer  
Diagnostics and Risk Assessment

Dear Dr. Chornokur:

On 7/21/2011, the Institutional Review Board (IRB) determined that your research meets USF requirements and Federal Exemption criteria as outlined in the federal regulations at 45CFR46.101(b):

(4) Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

As the principal investigator for this study, it is your responsibility to ensure that this research is conducted as outlined in your application and consistent with the ethical principles outlined in the Belmont Report and with USF IRB policies and procedures. Please note that changes to this protocol may disqualify it from exempt status. Please note that you are responsible for notifying the IRB prior to implementing any changes to the currently approved protocol.

The Institutional Review Board will maintain your exemption application for a period of five years from the date of this letter or for three years after a Final Progress Report is received, whichever is longer. If you wish to continue this protocol beyond five years, you will need to submit a continuing review application at least 60 days prior to the exemption expiration date. Should you complete this study prior to the end of the five-year period, you must submit a request to close the study.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

A handwritten signature in cursive script, reading "Janelle Perkins PharmD". The signature is written in black ink and is positioned above the printed name.

Janelle Perkins, PharmD, Chairperson  
USF Institutional Review Board



App. 2



August 24, 2011

Ganna Chornokur, PhD  
H. Lee Moffitt Cancer Center & Research Institute  
University of South Florida  
12902 Magnolia Drive  
Tampa, FL 33612

Dear Dr. Chornokur:

The Scientific Review Committee (SRC) has reviewed your response to its critique of amendment version dated 07/05/2011 to your research protocol entitled, "Comprehensive Population-Specific Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment" (MCC 16701). The revised amendment version dated 08/15/2011 is approved as written for use at the Moffitt Cancer Center pending approval of the Institutional Review Board (IRB).

The use of tissue and data for research purposes is essential to advancing the scientific field; however because of safety, privacy, and security reasons, such research use is highly regulated by the federal government. Accordingly, the use of such tissue must be in accordance with the applicable Institutional Review Board (IRB) policy. Any changes or deviations that you wish to carry out on your approved study must be reported to the SRC Coordinator for determination of whether additional Scientific Review Committee (SRC) and IRB approvals are needed. This approval does not guarantee access to tissue samples.

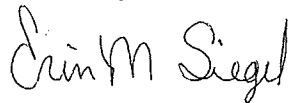
It is your responsibility to ensure that all Moffitt staff (pathology, tissue core, data management, etc.) are informed and aware of the details of the project. The committee encourages the use of in-services for those projects that are complex or require special attention.

All changes made to protocols approved by the SRC must be submitted to the Protocol Review and Monitoring System office. Changes made to the protocol document require SRC review and approval. Minor changes (i.e. changes to personnel, non-scientific changes, changes that do not affect patient participation) will be expedited through the SRC review process.

If this project is not being managed by the Clinical Trials Office or Clinical Research Unit, then it is your responsibility to follow through with all requirements for submission to the IRB. All IRB approvals are required to be documented in Oncore, and all associated regulatory documentation (signed applications, IRB approval letters and IRB approved consent forms, etc.) are to be saved in the appropriate study folder in the e-binders directory at J:\ebinders.

Oncore is the Cancer Center's mechanism for submission and review of materials requiring Scientific Review (SRC) and Protocol Monitoring (PMC). If you need access to Oncore, please contact Jeryl Madden, Oncore Administrator, at 745-6964 for assistance.

Sincerely,

A handwritten signature in cursive script that reads "Erin M Siegel".

Erin Siegel, PhD, MPH  
Chair, Scientific Review Committee

**SPECIFIC AIMS:**

The American Cancer Society estimates that about 240,890 new cases of prostate cancer (PCa) occurred in the US during 2011 and 33,720 men died due to this disease. While PCa remains the most common type of solid malignancy, and the second-leading cause of all cancer death in men, African American men (AAM) have 1.6 times the incidence, and more than twice the mortality rate of PCa, compared to European American men (EAM) [1]. Although the reason(s) for this striking racial disparity are not well understood, recent research have identified the overactive survival mechanisms, that may predispose AAM to a more biologically aggressive disease. Despite advances in the screening, early detection and treatment of PCa, the mortality and morbidity burden in AAM from the disease remains high. With the current uncertainty and complexity with regard to timing and frequency of screening for high risk populations, there is a pressing need for PCa control beyond early screening and detection. It is well recognized that the most promising approach to cancer control is a national commitment to prevention. Notably, PCa constitutes an ideal chemopreventive target due to existence of well defined premalignant lesions, as well as a generally long period of tumor dormancy in the localized stage [2;3].

Epidemiological and laboratory studies have demonstrated that several plant-derived botanicals influence multiple biochemical and molecular cascades that inhibit mutagenesis, proliferation, induce apoptosis and suppress the formation and growth of human cancers, modulate genetic and epigenetic pathways, thus targeting several hallmarks of carcinogenesis. Among these promising agents, curcumin (diferuloylmethane, an Indian dietary spice) was found to delay initiation and slow down PCa progression in the epidemiological and molecular studies [4-6], and animal models [7;8]. Curcumin exerts its multitargeted anticancer activity on myriad of cellular pathways that are essentially antiproliferative and proapoptotic [9-10]. Curcumin has been shown to inhibit proliferation in several prostate cancer cell lines, such as LNCaP, DU-145, and PC3 [9;11]. Curcumin has also been shown to induce cell cycle arrest in G1/S [12] and G2/M [13] phases in LNCaP and PC3. Additionally, curcumin induces apoptosis in LNCaP, DU145 and PC3 [12;14]. Curcumin inhibits NFkB activity in LNCaP and DU145 [14]. Lastly, curcumin inhibited invasiveness and reduced the MMP2 and MMP9 expression in DU145 [7]. Interestingly, AA-derived PCa tumors were found to have over expressed signaling receptors (including AR and EGFR) [15-16] and significantly greater proliferation rates can be observed in AA-derived prostate tumors relative to EA-derived tumors. Similarly, key anti-apoptotic protein BCL-2 is up-regulated [17-18], NFkB pathway is more active [19] in AAM-derived PCa tumors, whereas elevated expression of MMP9 has also been observed, increasing their invasive capacity [20]. These characteristics observed in AA-derived prostate cancer cell lines compared to EA-derived cell lines suggest that AAM and EAM PCa tumors treated with curcumin may exhibit different proliferation rates, demonstrate different cell cycle profiles, apoptosis rates, NFkB activities, and invasion capacities. Since curcumin specifically targets these molecular processes critical to prostate carcinogenesis, at the public health level, these data provide first clues that AAM may have even better response to curcumin in terms of PCa chemoprevention. However at present, the effect(s) tumor biological differences exert on the chemoprevention of PCa with curcumin in AAM are unknown. Given the increased burden of PCa in AAM and potentially high effectiveness of PCa chemoprevention in reducing the disease burden, there is a dire need to establish and compare the effectiveness of PCa chemoprevention with curcumin in AAM and EAM, in order to inform the design of future chemopreventive trials in this exceptionally high risk population. With the availability of several commercially standardized formulations of curcumin and excellent safety profile, curcumin is an attractive compound to be tested for PCa chemoprevention. Our long term goal is to optimize the effectiveness of chemopreventive intervention against PCa in AAM and EAM. The objective of the proposed work is to determine whether chemopreventive effects of curcumin differ in AAM and EAM PCa cell lines. Our central hypothesis is that chemopreventive effects of curcumin, as measured by the changes in the main carcinogenic events - cell proliferation, cell cycle/ apoptosis and invasion/ metastasis - differ in AAM and EAM due to the underlying differences in tumor biology. We propose the following specific aim to test our hypothesis.

**Specific aim 1** :To compare the chemopreventive effects of curcumin, as measured by the changes in the main carcinogenic events - cell proliferation, cell cycle/ apoptosis and invasion/ metastasis, in the well-characterized PCa cell lines, derived from AAM and EAM prostate tumors. Our successfully completed project is expected to contribute to evidence-based, clearly documented chemopreventive effects of curcumin in AAM and EAM derived PCa cell lines, as measured by the markers of cell proliferation, cell cycle/ apoptosis and invasion/metastasis. These mechanistic data are of a critical importance for designing a chemopreventive clinical trial of curcumin in men with PCa. Because AAM consistently carry increased burden of PCa, evidence based, specifically tailored chemopreventive intervention would offer tremendous public health benefit not only to that particular racial group, but to racially and ethnically diverse U.S. population as a whole.

# App. 4

**SPECIFIC AIMS.** Both obesity (Body Mass Index (BMI)  $\geq 30$ ) and prostate cancer (PCa) constitute significant public health problems, for which the disparities between African American men (AAM) and Non-Hispanic European men (EAM) are evident. AAM are 10% more likely to be obese [1], 60% more likely to be diagnosed with, and 145% - to die from [2], PCa compared to their EAM counterparts. While obesity is an established risk factor for PCa biochemical recurrence and/or post treatment progression [3], the specific role of obesity in PCa risk is under investigation. Obesity as a physiological state is characterized by significant alterations in the individual's hormonal profile [4]. Since PCa is a hormonal disease, it appears biologically plausible that obesity affects PCa risk, possibly through the altered tumor growth enabled by the hormonal imbalances [5]. This effect may be even more pronounced in AAM since their PCa tumors tend to demonstrate more aggressive biological behavior at baseline [6-12]. Epidemiological studies have linked obesity to a slight decrease in the incidence of a low-grade (non-lethal) PCa and an increase in the incidence of an aggressive (lethal) disease [13-14]. A few studies [15-16] have linked obesity to more aggressive PCa in AAM specifically, suggesting involvement of obesity in PCa disparity. However, to date, the mechanistic biology studies aimed to elucidate the effects of obesity on PCa risk in AAM and EAM are lacking.

Our recent observation comparing AAM and EAM with and without PCa indicates that obese AAM (odds ratio (OR) 1.06;  $p=0.022$ ) but not EAM (OR 0.98;  $p=0.332$ ) have slightly elevated overall PCa risk [Chornokur et al, appendices 1]. We further observed that AAM homozygous for the minor allele of the single nucleotide polymorphism (SNP) rs7501939 in the *HNF1B* gene have increased PCa risk (OR 2.14;  $p=0.0103$ ) over 2-fold. Interestingly, the excessive risk is not observed in the non-obese AAM (OR 1.09;  $p=0.763$ ) or EAM of any BMI ( $p>0.05$ ). *HNF1B* encodes a member of a transcription factor superfamily that is expressed in the kidneys, pancreas and prostate. As such, it modulates key cellular processes, such as proliferation. Genetic variation in the *HNF1B* was previously associated with PCa [17-18] and type II diabetes [18], however the underlying mechanistic pathway(s) remain elusive. Taken together, these data suggest that obesity may be a PCa risk factor in AAM, and the extent of risk is determined by the individual's genetic variation. The goal of our project is to understand the joint impact of germline genetic variations and obesity on prostate tumor biology and PCa risk in AAM and EAM. We hypothesize that select genetic variation, when combined with the environment of obesity, influences PCa risk by impacting key cellular processes relevant to the tumor biology. We propose the following specific aims to test our hypothesis:

1. To elucidate the effects of obesity on the gene expression profiles in AAM and EAM PCa tumors.

Approach. PCa tumors derived from obese and non-obese AAM and EAM, matched for age and Gleason grade will be profiled for expression of select genes using the NanoString Gene expression assays.

2. To test the functional significance of the selected genes *in vitro* and *in vivo*.

Approach. A. AAM and EAM derived PCa cell lines will be grown with and without the environment of obesity (sera from mice with diet-induced obesity). The genes of interest will be knocked down and the effects of the knock-down on the growth will be studied. B. Cell lines with the most promising genes from the sub-aim A will be injected into nude mice (normal weight and obese) to monitor the effect of the knock-down on the tumor growth *in vivo*.

3. To link obtained data to PCa risk through variation in the germline genome.

Approach. We will genotype the genes of interest using germline DNA from AAM and EAM cases and controls. We will use statistical approaches to determine whether genetic variation correlates with PCa risk.

Significance. Although AAM consistently carry increased PCa burden, the etiology of the disparity is unclear. AAM are also more likely to be obese, compared to their EAM counterparts. Thus, understanding the specific role of obesity in PCa risk is crucial for efficient development of individualized PCa risk estimation in AAM and EAM; selection of the most appropriate treatment modalities; estimating the risk of progression; advancing knowledge of the tumor biology in AAM and EAM; and developing targeted risk reduction interventions and informing the policies on healthy lifestyle and PCa prevention in diverse populations. Innovation. Our work is innovative, comprehensive and contemporary in that we will examine, within a single integrative approach, how obesity alters gene expression in PCa tumors of AAM and EAM; how these alterations affect PCa growth in AAM and EAM; and whether germline genetic variation in those genes affects PCa risk in AAM and EAM. Unlike the non-candidate driven studies (such as GWAS) that cannot, on its own, elucidate the functional significance, we propose functionally relevant genetic variation to be incorporated in the risk prediction. Future directions.

Guided by our findings, we plan to launch a clinical preventive study aimed to validate the results in a larger cohort of AAM and EAM, improve the proposed approach, and test the chemopreventive and/or lifestyle interventions targeted towards specific tumor and genetic features in AAM and EAM. In addition, our proposed model can be applied to study gene-environmental interactions in virtually any other malignancy.

App. 4a

**PROGRAM CONTACT:**

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**SUMMARY STATEMENT**  
( Privileged Communication )

*Release Date:* 04/03/2013

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*Application Number:* 1 R21 CA179659-01

Principal Investigator

CHORNOKUR, GANNA

Applicant Organization: H. LEE MOFFITT CANCER CTR & RES INST

*Review Group:* ZRG1 OBT-M (55)

Center for Scientific Review Special Emphasis Panel

PAR Panel: Cancer Health Disparities/Diversity in Basic Cancer Research

*Meeting Date:* 03/18/2013

*RFA/PA:* PAR12-094

*Council:* MAY 2013

*PCC:* F3CD

*Requested Start:* 07/01/2013

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*Project Title:* Obesity and prostate cancer risk in Black and White men: a functional approach

*SRG Action:* Impact Score: 39    Percentile: 30 #

*Next Steps:* Visit [http://grants.nih.gov/grants/next\\_steps.htm](http://grants.nih.gov/grants/next_steps.htm)

Human Subjects: E4-Human subjects involved - Exemption #4 designated

Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

Project Year	Direct Costs Requested	Estimated Total Cost
1	150,000	252,750
2	125,000	210,625
<hr/> TOTAL	<hr/> 275,000	<hr/> 463,375

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ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

**1R21CA179659-01 CHORNOKUR, GANNA**

**RESUME AND SUMMARY OF DISCUSSION:** The purpose of this study is to further understand the role obesity plays in prostate cancer (PCa) aggressiveness in African American men (AAM) compared to European American men (EAM), by exploring gene expression profiles of obese and non-obese men from each race and testing functional significance of the identified genes. The study is pertinent to cancer health disparities. Strengths are: biological relevant concept under consideration; state of the art technology; access to a good pool of human samples already collected; potential translational relevance; the cumulative expertise of the investigative team. Weaknesses that dampened enthusiasm include: selection of chemokines may not be relevant to PCa; ambitious nature of the experimental design for a two year time frame; completion of Aims 2 & 3 is dependent of success of Aim 1; and normal adjacent tissue will not be compared to tumors. Several reviewers expressed concern with the minimum percent effort from senior collaborators and uncertain that the applicant has enough experience to drive the project. Most members of the study section agreed that these weaknesses diminish the impact of the proposal to the PCa and cancer health disparities fields and were score driving. However, few were more enthusiastic because of the proposal's translational potential.

**DESCRIPTION (provided by applicant):** Both obesity (Body Mass Index (BMI)  $\geq 30$ ) and prostate cancer (PCa) constitute significant public health problems, for which there are persistent disparities between African American men (AAM) and Non-Hispanic European men (EAM). Obesity as a physiological state is characterized by significant alterations in the individual's hormonal profile. Since PCa is a hormonal disease, it appears biologically plausible that obesity affects PCa risk, possibly through the altered tumor growth enabled by the hormonal imbalances. This effect may be even more pronounced in AAM since their PCa tumors tend to demonstrate more aggressive biological behavior at baseline. Indeed, a few studies have linked obesity to more aggressive PCa in AAM specifically, suggesting involvement of obesity in PCa disparity. However, to date, the mechanistic biology studies aimed to elucidate the effects of obesity on PCa risk in AAM and EAM are lacking. In concordance with the published literature, our recent preliminary data suggest that obesity may be a PCa risk factor in AAM, and the extent of risk is determined by the individual's genetic variation. The goal of our project is to understand the joint impact of germline genetic variations and obesity on prostate tumor biology and PCa risk in AAM and EAM. We hypothesize that select genetic variation, when combined with the environment of obesity, influences PCa risk by impacting key cellular processes relevant to the tumor biology. To test this hypothesis, a functional integrated approach is proposed, that utilizes prostate tumor biology as a starting point. In the Aim 1, we will investigate whether there are differences in the gene expression profiles of obese and non-obese men in each race. In the Aim 2, we will test the functional significance of the identified genes in vivo (cell lines) and in vitro (mice). Finally, in the Aim 3 we propose to link obtained data to PCa risk through variation in the germline genome. Significance. Understanding the specific role of obesity in PCa risk is crucial for efficient development of individualized PCa risk estimation in AAM and EAM; selection of the most appropriate treatment modalities; estimating the risk of progression; advancing knowledge of the tumor biology in AAM and EAM; and developing targeted risk reduction interventions. Innovation. Our work is innovative, comprehensive and contemporary in that we propose functionally relevant genetic variation to be incorporated in the risk prediction. Future directions. Guided by our findings, we plan to launch a clinical preventive study aimed to validate the results in a larger cohort of AAM and EAM, improve the proposed approach, and test the chemopreventive and/or lifestyle interventions targeted towards specific tumor and genetic features in AAM and EAM. In addition, our proposed model can be applied to study gene-environmental interactions in virtually any other malignancy.

**PUBLIC HEALTH RELEVANCE:** The proposed research is relevant to public health because its overarching goal is to reduce the prostate cancer burden on society, by understanding the role of obesity and germline genetic variation in prostate cancer risk in men of African and European descent.

Thus, the proposed research is relevant to the NCI's mission of supporting research with respect to the prevention of cancer.

#### **CRITIQUE 1:**

Significance: 2  
Investigator(s): 2  
Innovation: 3  
Approach: 5  
Environment: 2

#### **Overall Impact:**

The relationship between obesity and predisposition of other diseases is becoming more relevant as the number of investigations increase. This proposal seeks to study the relationship between obesity, prostate cancer (PCa) and genetic variation. The investigators will use in vitro and in vivo approaches to explore gene expression and translate these findings back to human germline variations to determine risk prediction in African-American and European-American men. This proposal is highly significant and innovative partly justifying the level of enthusiasm.

#### **1. Significance:**

##### **Strengths**

- Obesity is a significant public health problem. The relationship between obesity and PCa is significant and highly relevant.

##### **Weaknesses**

- No major weaknesses noted.

#### **2. Investigator(s):**

##### **Strengths**

- Dr. Chornokur has put together a highly qualified Research team.
- Inclusion of biostatistician and the needed expertise is supported.

##### **Weaknesses**

- No major weaknesses noted.

#### **3. Innovation:**

##### **Strengths**

- A true translational approach to in vitro and in vivo gene expression exploration and the correlating these findings back to human germline variations to determine risk prediction in African-American and European-American men.
- The inclusion of a "gene-environment" analysis with in vitro, in vivo and risk determination.

##### **Weaknesses**

- General SNP profiling of AAM versus EAM men, not novel.

#### **4. Approach:**

##### **Strengths**

- Candidate-driven approach following gene expression analysis, then determination of functional relevance in vitro and vivo is novel.

##### **Weaknesses**

- The use of genomics to explore general African-American versus European American is not novel. Exploration of those genes selected or re-sequencing of those genes selected would give increased value to SNP selection and profiling.

- Obesity induces inflammation; the modulation of inflammatory proteins in human cell lines may not be relevant in a rodent xenograft model.

**5. Environment:**

**Strengths**

- The environment is sufficient and adequate to sustain the completion of proposed goals.

**Weaknesses**

- No major weaknesses noted.

**Protections for Human Subjects:**

Acceptable Risks and/or Adequate Protections

- Falls under exempted study.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

- Not Applicable

**Inclusion of Women, Minorities and Children:**

G3A - Only Men, Acceptable

**Vertebrate Animals:**

Acceptable

- The five points well addressed.

**Biohazards:**

Not Applicable

**Applications from Foreign Organizations:**

Not Applicable (No Foreign Organizations)

**Select Agents:**

Not Applicable (No Select Agents)

**Resource Sharing Plans:**

Acceptable

**Budget and Period of Support:**

Recommend as Requested

**CRITIQUE 2:**

Significance: 4

Investigator(s): 4

Innovation: 3

Approach: 5

Environment: 4

**Overall Impact:**

The applicant proposes to evaluate the joint impact of genetic variation and obesity on prostate cancer tumor biology and prostate cancer risk in African American and European American men. The application might lead to improved predication of prostate cancer risk as well as clinical and lifestyle interventions to reduce the risk of prostate cancer. The application is high risk and novel – and if successful, can establish a new paradigm for conducting research on gene-environment interactions.



The primary project seeks to accomplish broad goals, but lacks a methodical approach that can lay the groundwork for future research, reducing its impact.

### **1. Significance:**

#### **Strengths**

- The role of obesity in prostate cancer risk is unknown; African American men have a higher risk of both; hormone imbalances represent a plausible mechanism by which obesity may impact prostate cancer risk.
- Preliminary data show possible interaction between genes and environment, thus there is a need for further examination of this topic.
- The applicant proposes to focus on functionally relevant genetic variation to ultimately improve risk prediction and direct clinical and lifestyle interventions.
- Data collected from this study can be used to conduct individualized prostate cancer risk assessments for African American and European American men, and to advance the knowledge of prostate cancer tumor biology.

#### **Weaknesses**

- Sample sizes are too small to draw solid conclusions; thus even if aims are achieved, the findings will need to be confirmed in larger, more controlled, studies.

### **2. Investigator(s):**

#### **Strengths**

- Dr. Chornokur is a post-doctoral scientist at Moffitt Cancer Center and will contribute significant effort (75%) to the project.
- The investigator is developing a track record of research in prostate cancer and her research has been recognized at AACR Health Disparities conferences.

#### **Weaknesses**

- An important concern is the low percent effort contributed by the senior investigators, most of who will participate in project meetings, deliver presentations, and prepare manuscripts.
- An additional weakness is the lack of expertise across the broad range needed for the diverse aspects of the study – more expertise in population sciences, less in animal models.

### **3. Innovation:**

#### **Strengths**

- The project is highly innovative – the approach relies on the use of tumor biology as a starting point for identifying candidate genes responsible for prostate cancer risk. The applicant plans to apply what is learned from humans to animal models.

#### **Weaknesses**

- The project is perhaps overly ambitious– as it is unclear whether the findings from humans can be applied to animal models. Alternative approaches are not fully considered.

### **4. Approach:**

#### **Strengths**

- The approach involves a combination of human and animal studies.

#### **Weaknesses**

- The project is ambitious and may not be readily accomplished in the allotted timeframe. It is preferable to limit the scope of this project, in a more rigorously controlled manner, than to conduct a broader less rigorous study, as proposed.
- The relatively small sample size, limited phenotype data, and somewhat simplified analysis plan means that the strategy proposed may be unable to detect true associations that may exist.

### **5. Environment:**

**Strengths**

- Moffitt Cancer Center has sufficient resources and facilities to conduct the proposed study.
- The study will use retrospectively collected samples from African American and European American men.

**Weaknesses**

- It is unclear how much dedicated laboratory space the applicant has, and the commitment of Moffitt Cancer Center to provide her with space, as the applicant has served as a post-doctoral scientist for 4 years.

**Protections for Human Subjects:**

Acceptable Risks and/or Adequate Protections Data and Safety

- Anonymous samples already collected- Exemption #4

Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

**Inclusion of Women, Minorities and Children:**

G3A - Only Men, Acceptable

M1A - Minority and Non-minority, Acceptable

C3A - No Children Included, Acceptable

**Vertebrate Animals:**

Acceptable

**Biohazards:**

Not Applicable (No Biohazards)

**Applications from Foreign Organizations:**

Not Applicable (No Foreign Organizations)

**Select Agents:**

Not Applicable (No Select Agents)

**Resource Sharing Plans:**

Acceptable

**Budget and Period of Support:**

Recommend as Requested

**CRITIQUE 3:**

Significance: 1

Investigator(s): 1

Innovation: 2

Approach: 3

Environment: 1

**Overall Impact:**

This proposal seeks to determine whether genomic anomalies, including differentially expressed genes and genetic susceptibilities, coupled with obesity may help explain disparities in prostate cancer among African-Americans. They are also interested in understanding how differential expression of "obesity" genes may influence prostate cancer development using African-American and European prostate cancer cell lines. Their hypothesis, suggest that "obesity" genes combined with obesity may explain

prostate cancer onset, progression, and more importantly health disparities. This goal is well supported by published reports and preliminary data and is consistent with NIH's provocative question regarding the link between obesity and cancer. There is some accumulating data suggesting that obesity may be related to prostate cancer risk, especially aggressive prostate cancer. The American Cancer Society and the National Institute of health are particularly interested in the link between obesity and cancer, which makes the proposed project a timely one. Adding the genomic and disparity elements make it particularly intriguing and novel, conceptually. This proposal offers a paradigm shift away from GWAS studies which sought to understand the genetic architecture of various cancers, leaving us with the identification of targets without any information about their biological importance. Instead, expression analysis of obese versus non-obese genes in AAM and EAM are used to guide functional assays in vitro as well as genetic susceptibilities within a human population study. The applicant 3 1st authorships and 4 co-publications, and has assembled a stellar multi-disciplinary research team with documented evidence of collaborations. The statistician and oncologist are highly productive. This well written proposal has a high likelihood of helping to refocus basic scientist, epidemiologist, and biosystems biologists in terms of understanding genetics and obesity in relation to prostate cancer and other malignancies. If successful, it will help to make better recommendations in terms of readily modifiable risk factors. Human tissue specimens and germ-line DNA will be used in the proposed project, but a biohazard safety protocol was not addressed in the proposal.

## **1. Significance:**

### **Strengths**

- In the population where 3.5 out of 10 adults are overweight or chronically overweight and 1 and 6 men are diagnosed with prostate cancer, understanding the role of obesity in PCA risk is crucial for efficient control of this disease.
- This proposal will inform future obesity-genetic studies in virtually all malignancies.
- Improve our understanding on the role of the obesity-genetic axis on prostate cancer health disparities.
- Data will be useful to eventually provide medical and public health value to aid with prostate cancer risk assessment as well as intervention strategies.
- Future studies by the research team will use the results to inform future policies on healthy lifestyle and prostate cancer prevention in diverse populations.

### **Weaknesses**

- None noted

## **2. Investigator(s):**

### **Strengths**

- The applicant is a Post-doc fellow at Moffitt Cancer Center and Research Institute.
- He has 7 publications of which 3 are 1st authorships and 2 submitted manuscripts.
- The applicant is member of a multidisciplinary team with 4 co-publications.
- Dr. Kumar is an expert in cancer chemoprevention and obesity, clinical trials, translational research and cancer health disparities, particularly PCa disparity.
- Dr. Jong Park is an expert in molecular and genetic epidemiology focusing on PCa. He is actively involved in PCa tumor biology research, as well as characterization of genetic and epigenetic risks in PCa. Dr. Park is building a model to predict metastatic progression of PCa, including the influence of obesity.
- Dr. Catherine Phelan is an expert in molecular genetics. Dr. Phelan's research interests include cancer health disparities in African American men with PCa and Latin American women with breast cancer.
- Dr. Hui-Yi Lin is an expert in genetic and biomedical statistics who will provide help with the data analysis. Documented collaboration with Dr. Jong Park.

### **Weaknesses**

- None noted.

### **3. Innovation:**

#### **Strengths**

- Looking at the link between prostate cancer and obesity is novel, especially as it relates to prostate cancer health disparity research.
- Suggest a paradigm shift away from GWAS and back to a candidate gene approach.

#### **Weaknesses**

- Overall methods are pretty common.

### **4. Approach:**

#### **Strengths**

- Tumor expression profiles from pre-clinical studies in Aim 2 will be saved for future studies.
- Availability of survival data among the cases will permit future exploratory studies.
- Seeks to use a state-of the art genomic approach that is suitable for archival tissue.
- State of the art equipment for gene expression analysis.

#### **Weaknesses**

- Not clear how race/ethnic-specific genes will be selected in Aims 1-3.
- For Aim 2, no justification for focusing on the top 5 genes selected in Aim 2. How will the selected genes be prioritized?
- Pathway analysis tools are ignored in Aim 1. This seems like a missed opportunity to find race-specific genes/pathways responsible for obesity induced prostate cancer.
- Seems like a missed opportunity to ignore gene-gene interaction effects in Aim 3.
- Aim 1, Not sure what percent of subjects would have a BMI 25-29.9. Would these individuals be placed in the "high" or "low" risk group?
- Aim 1, analysis gene expression separately for each racial/ethnic group is fine but it seems like using AAMs to select for high-risk genes should be considered as well. In this regard, genes that are over- or under-expressed relative to EAM may serve as high-risk markers.
- Aim 2, budget limitations are acknowledged and understood. However, failure to tease out the cancer related genes from the surrounding "normal" tissue may dilute the capacity to finding meaningful relationships.
- Aim 1, not clear what % change in means between "obese vs. non-obese" or "AAM vs. EAM" are needed for the results to be meaningful. Of course for the racial ethnic comparisons, one would have to "subtract out" or adjust for normal tissue expression profiles.
- Aim 3, not clear what strategy if any will be used to prioritize and select 205 SNPs.

### **5. Environment:**

#### **Strengths**

- Outstanding research environment. All resources for the proposed project are readily available.

#### **Weaknesses**

- None noted.

### **Protections for Human Subjects:**

Acceptable Risks and/or Adequate Protections

- adequately addressed

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

### **Inclusion of Women, Minorities and Children:**

G3A - Only Men, Acceptable

M1A - Minority and Non-minority, Acceptable  
C3A - No Children Included, Acceptable

**Vertebrate Animals:**

Acceptable

- Adequately addressed

**Biohazards:**

Unacceptable

- Human tissue specimens will be analyzed; however, no biohazard safety methods were noted.

**Applications from Foreign Organizations:**

Not Applicable (No Foreign Organizations)

**Select Agents:**

Not Applicable (No Select Agents)

**Resource Sharing Plans:**

Acceptable

- Adequately addressed.

**Budget and Period of Support:**

Recommend as Requested

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

**PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE.** Use of anonymous and retrospectively collected for another study, their use is justified. Exemption 4 is applicable.

**VERTEBRATE ANIMAL (Resume): ACCEPTABLE.** The applicant has adequately described the use of mice in the proposed studies. Both the number and experimental details are well justified and clearly presented.

**COMMITTEE BUDGET RECOMMENDATIONS:** The budget was recommended as requested.

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# Ad hoc or special section application percentiled against "Total CSR" base.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at

<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html>.

The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see [http://grants.nih.gov/grants/peer\\_review\\_process.htm#scoring](http://grants.nih.gov/grants/peer_review_process.htm#scoring).

1 R21 CA179659-01  
CHORNOKUR, G

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ZRG1 OBT-M (55)

## MEETING ROSTER

**Center for Scientific Review Special Emphasis Panel  
CENTER FOR SCIENTIFIC REVIEW  
PAR Panel: Cancer Health Disparities/Diversity in Basic Cancer Research  
ZRG1 OBT-M (55) R  
March 18, 2013 - March 19, 2013**

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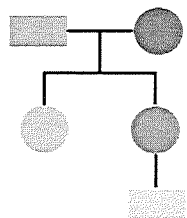
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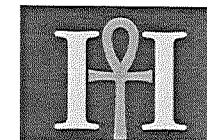
# Risk of gastrointestinal cancers in female BRCA1 and BRCA2 mutation carriers



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**Background:** It is estimated that 10-15% of all cancers are directly attributed to specific genetic alterations that are passed among generations and are known as "cancer syndromes". Among the latter, Hereditary Breast and Ovarian Cancer Syndrome (HBOC) that is characterized by deleterious mutations in the BRCA1 and BRCA2 genes is the most prominent and described. While the increased risk of colorectal and liver cancer was seen in BRCA1 carriers, while increased risk of gallbladder/bile duct cancers were attributable to BRCA2. However, no significant associations were reported in other studies, and thus the data is inconclusive. Because BRCA1 and BRCA2 mutation carriers may constitute up to 4% in certain ethnic groups (notably, Ashkenazi Jews), accurate penetrance estimates are important for the counseling and management of cancers in this particularly vulnerable group.

**Study goal and methods:** In this work, we thought to estimate the incidence of GI cancers in 4536 female BRCA1 and BRCA2 mutation carriers that were followed for a mean of 4 years (range 1 month to 11 years). Incidence rates were compared with population-specific incidence rates and relative risks (RRs) to carriers together with 95% confidence intervals (CIs) were estimated by use of a maximum likelihood approach.

**Study population:** Eligible women who carried deleterious BRCA1 or BRCA2 mutations were recruited at the participating study sites from United States, Canada, Europe and Israel from 1992 to 2003. Written informed consent was obtained and the study was IRB approved. The members of the cohort were followed up until: 1. the date of completion of the follow-up questionnaire; 2. the development of cancer; 3. age 75 years; or 4. death. All cancers were confirmed by pathology reports. The majority of women were of a Non-Hispanic White (Caucasian) ancestry.

**Results:** There were 4559 women (xx carried BRCA1, xx carried BRCA2 mutation, and 37 (0.4%) carried both) with the mean age at entry 47.3 years (range 30-74 years). The women were observed for 4 years. 13 GI cancers developed in BRCA1 (mean age 53.5 years), and 6 – in BRCA2 mutation carriers (mean age 57.3 years). Detailed characteristics of BRCA1/2 female mutation carriers who developed GI cancers is presented in Table 1. The RRs for colon, rectal, stomach, pancreatic and gallbladder/bile duct cancers, by the BRCA1 or BRCA2 carrier status are presented in Figure 1. For comparison, the RRs observed in the general population are shown as 1.

Pt#	GI cancer	Stage	Age at dx	BRCA1 or BRCA2	Mutation	Prior cancer(s)
1	Liver			BRCA1	exon 16 C4808G	breast
2	Colon			BRCA1	exon 11 1559insA	none
3	colon			BRCA1 and BRCA2	exon 16 5083del19, exon 11 5946delCT	breast
4	Rectal			BRCA1	exon 11 Q494X	Ovarian, HNC
5	Colon			BRCA1	exon 11 4184del4 (delTCAA)	none
6	Rectal			BRCA2	exon 11 6024delTA (1943 ter)	Breast, ovarian
7	Bile duct			BRCA2	missing	ovarian
8	Colon			BRCA1	exon 15 4693delAA	Breast, skin
9	Colon			BRCA1	exon 20 5382insC (1829 ter)	Breast, ovarian
10	colon			BRCA2	exon 11 5804del4	Breast, skin
11	Rectal			BRCA1	exon 11 1048delA	Breast, ovarian
12	rectal			BRCA1	exon 11 Q491X	none
13	colon			BRCA1	exon 20 5382insC (1829 ter)	breast
14	pancreas	????	????	BRCA1	??????	??????
15	pancreas	????	????	BRCA1	??????	??????
16	pancreas	????	????	BRCA1	??????	??????
17	pancreas	????	????	BRCA2	??????	??????
18	pancreas	????	????	BRCA2	??????	??????

Table 1. Detailed characteristics of BRCA1/2 mutation carriers who developed GI cancers.

**Conclusion:** our results establish important differences in the site-specific cancer risks, associated with BRCA1 and BRCA2 mutations. BRCA1 mutations are associated with the modestly increased risk of colon, rectum, colorectum, pancreatic, liver and gallbladder/bile duct cancers, while BRCA2 mutations are associated with increased risk of rectal, pancreatic and gallbladder/bile duct cancers, but decreased risk of cancers of colon and colorectum. Our results suggest decreased risk of stomach cancer in both the BRCA1 and BRCA2 female mutation carriers. However, additional research is needed to accurately document the site-specific penetrance estimates in these specific populations.

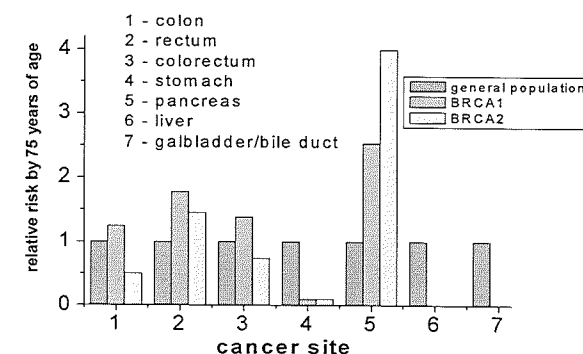
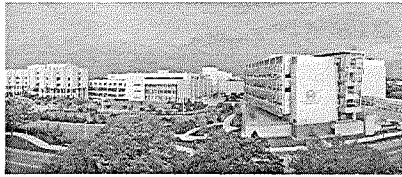


Figure1. The RRs for GI cancers by the BRCA1 or BRCA2 carrier status. The general population risk is taken as 1.

**Discussion:** Although we have observed an increase in the RRs of the most GI cancers in the BRCA1, and some GI cancers in the BRCA2 female mutation carriers, these increases were modest ranging from 1.25 (RR of colon cancer in BRCA1 carriers) to 4 (RR of pancreatic cancer in the BRCA2 carriers). Our findings are in agreement with, to date, the largest studies of cancers in BRCA1 and BRCA2 carriers by Thompson et al [1], Ford et al [2] and the Breast Cancer Linkage Consortium [3]. The authors have reported modest increases in pancreatic (BRCA1: RR=2.26; BRCA2: RR = 3.51), gallbladder and bile duct (BRCA2: RR=4.97), colon (BRCA1: RR=2.03) and liver (BRCA1: RR=4.06) cancer incidences, and possibly other abdominal/GI cancers. One of our intriguing findings is the absence of stomach cancers, although modestly increased stomach cancer RRs (1.1-4) were previously reported in BRCA1/2 mutation carriers [3-5]. However, the incidence of stomach cancer in white females in the developed countries is very low (about 4.5 per 100,000) and thus the absence of stomach cancers in the cohort of 5000 women comes at no surprise. Large cohorts of women and/or longer follow-up times may be needed to accurately estimate the RR of stomach cancer.

**References.** [1] Thompson et al. J. NCI. 94(18), 2002; [2] Ford et al. The Lancet 343, 1994; [3] Breast Cancer Linkage Consortium. J. NCI. 91(15), 1999; [4] Brose et al. J. NCI. 94(18), 2002; [5] Figer et al. BJC. 84(4), 2001.



# Risk Factors for Prostate Cancer in African American Men

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## Rationale

In the United States, African American/ Black men experience 1.6 times the incidence and 2.4 times the mortality rates of prostate cancer (PCa), and present with more advanced stages, as compared with Caucasian American men (Figures 1 and 2).

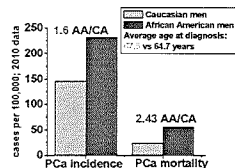


Figure 1. Incidence, mortality and age at prostate cancer diagnosis by race.

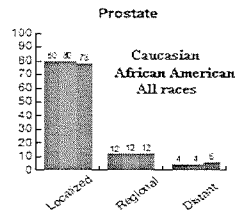


Figure 2. Stage at prostate cancer diagnosis by race (Jemal et al, 2010).

Population-wide screening with Prostate Specific Antigen (PSA) blood test has not been definitely proven to reduce prostate cancer mortality. Of approximately 1 million prostate biopsies performed every year in the U.S., around 70-80% are unnecessary, leading to increased anxiety and other potential side effects such as bleeding and infections in healthy men.

Hence, the need for an effective diagnostic and/or screening test, with higher sensitivity and specificity towards lethal prostate tumors has been recognized, specifically in the high risk population of African American / Black men. In an attempt to address this need, we have investigated known risk factors of prostate cancer, along with PSA levels, and risk of prostate cancer in African American / Black men.

## Subjects

Recruitment took place at Moffitt and community hospitals/physician practices, prior to initiation of treatment from 03/2008 till 03/2010. Eligible subjects self-classified as African American men and were 40-80 years old. Cases were defined as men having biopsy-confirmed prostate cancer. Controls were defined as men having no evidence of prostate cancer on biopsy and/or low PSA levels. All men signed an informed consent form and provided a blood sample for DNA, serum and plasma, risk factor questionnaire data and access to their medical records.

## Statistical Analysis

The following statistical analyses were performed.

- Descriptive statistics for discrete and continuous variables;
- Pearson's Chi-square test (Fisher's exact test) for independence;
- Wilcoxon rank-sum test for comparing cases and controls;
- Univariable and multivariable logistic regression models (with backward elimination and Hosmer-Lemeshow goodness-of-fit test);
- Spearman's correlation and Kendall's Tau correlation for age versus PSA, BPH and smoking.

## Results

A total of 35 cases and 70 controls were analyzed. Some continuous (Table 1) and categorical (Table 2) variables were statistically significantly different between cases and controls. Since the cases in our study were on average 8 years older than controls, we explored if there was a correlation between age versus PSA, and age versus BPH. Significant positive correlation between PSA and age ( $p=0.0045$ ), and BPH and age ( $p=0.0122$ ) was found; however, including age as a covariate had no effect on the univariate (Table 3) or multivariable (Table 4) logistic regression models. Hence, we have concluded that age difference did not significantly affect our results.

In the univariate model, six factors were associated with prostate cancer (Table 3). However, only PSA and BPH were significantly associated with prostate cancer in the multivariate logistic regression model (Table 4).

Table 1. Descriptive statistics for continuous variables.

Variable	Control mean (SD; n)	Case mean (SD; n)	p-value
Age at diagnosis, years	54.12 (9.13; 68)	59.40 (9.24; 35)	0.0071
Cigarettes smoked per day	10.29 (10.16; 34)	18.88 (13.79; 16)	0.011
PSA, (ng/ml)	1.24 (1.32; 31)	6.51 (8.14; 25)	<0.001

Table 2. Descriptive statistics for categorical variables.

Variable	Controls: % yes (number responded out of 70)	Cases: % yes (number responded out of 35)	p-value
Abnormal PSA results	5.5 (55)	94 (32)	<0.001
Diagnosis of BPH	23 (69)	59 (34)	<0.001
Diagnosis of PIN	10 (70)	89 (35)	<0.001
Diagnosis of prostatitis	2 (70)	26 (35)	<0.001

Table 3. Odds ratios and p-values from univariate logistic regression per diagnosis of prostate cancer\*.

Variable	Odds ratio point estimate; (95% CI)	P-value
Cigarettes smoked per day	1.066; (1.005, 1.130)	0.034
PSA, (ng/ml)	2.828; (1.672, 4.781)	<0.001
Positive PSA result (y/n), ever	350.998; (46.826, >999)	<0.001
Ever diagnosed with BPH	5.429; (2.041, 14.437)	<0.001
Diagnosed of PIN on biopsy	69.316; (16.007, 300.173)	<0.001
Ever diagnosed with prostatitis	20.180; (2.377, 171.351)	0.006

Table 4. Summary of the multivariate logistic regression per diagnosis of prostate cancer\*.

Variable	Standard Error	Odds Ratio (95% CI)
PSA, (ng/ml)	0.5474	4.340; (1.484, 12.689)
Ever diagnosed with BPH	1.2921	14.248; (1.132, 179.310)

\* Both models adjusted for age.

## Conclusions

In our study, PSA and BPH were significantly associated with prostate cancer in the multivariate logistic regression model. Despite the limitation of a small sample size, our data is in agreement to a recent report (Pettaway et al, 2011) in which African Americans with BPH were observed to have a much greater risk of developing prostate cancer than similar Caucasian men, highlighting differences in the biology of prostate cancer between populations. Additional studies with increased sample size are needed to further explore these differences.

## References

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Pettaway CA et al. BJU International. 2011;108(8):1302-8.

## Acknowledgements

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# Variation in the metabolism related genes and obesity may contribute to prostate cancer disparity in African American men

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## Rationale

In the United States, African American/ Black men are 60% more likely to be diagnosed with, and 140% more likely to die from, prostate cancer compared to their European American/ White counterparts (Figure 1). Despite significant prostate cancer burden in this exceptionally high risk population, the reasons for the disparity have not been elucidated, although appear to have genetic component. We investigated the association between selected functional polymorphisms (SNP) in the metabolism-related genes and the risk of prostate cancer in African American/ Black and Non-Hispanic White men. We further explored if these associations varied by body mass index (BMI) in the two races.

## Methods

Recruitment took place at the various hospitals in Tampa Bay prior to initiation of treatment. Eligible subjects self-classified as Non-Hispanic Black or White men and were 30-80 years old. Cases were defined as men having biopsy-confirmed prostate cancer. Controls were defined as men having no evidence of prostate cancer on biopsy and/or low PSA levels. All men signed an informed consent form and provided a blood or saliva sample for DNA, risk factor questionnaire data and access to their medical records.

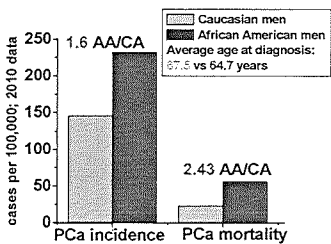


Figure 1. Prostate cancer incidence and mortality by race

Table 1. SNPs investigated in this study

SNP	Gene
rs4430796	<i>HNF1B</i>
rs7501939	<i>HNF1B</i>
rs1859962	<i>HNF1B</i>
rs10993994	<i>MSMB2</i>
rs822396	<i>ADIPOQ</i>
rs4263970	<i>EPHB2</i>
rs4612601	<i>EPHB2</i>
rs4962416	<i>CTBP2</i>
rs627839	<i>RNASEL</i>
rs2070874	<i>IL4</i>

## SNP selection

1. Previously reported being associated with prostate cancer in Black and White men;
2. Genes related to metabolism pathways;
3. At least some evidence for functional significance;
4. Minor allele frequency (MAF) at least 15% in either Black or White men;

The following 10 SNPs in 7 genes were selected (Table 1).

## Statistical analyses

Descriptive statistics was performed for discrete and continuous variables. The dominant, recessive and additive age-adjusted unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) fitted for all men combined, for each race and stratified by BMI, height and smoking habits within each race. Significance was defined as the p-value <0.05.

## Results

DNA samples from 259 (136 cases and 123 controls) Black and 269 (147 cases and 142 controls) White men were analyzed, yielding a total sample size of 528 men. White men were matched to Black men by case status, age, Gleason score and PSA. Significant findings are shown in the Table 2.

Table 2. SNPs associated with prostate cancer by race, controlled for age. NS= non significant.

SNP gene	Black men OR (CI); p-value	White men OR (CI); p-value	Stat. model
Rs7501939C <i>HNF1B</i>	1.56 (1.08-2.27); 0.0193	NS	Additive
rs4430796G <i>HNF1B</i>	0.57 (0.34-0.97); 0.0383	NS	Dominant
Rs4962416 T <i>CTBP2</i>	NS	1.69 (1.02-2.8); 0.0415	Dominant

Further analysis revealed that obesity in Black men was positively associated with prostate cancer. The excessive risk associated with obesity was modest, albeit statistically significant (OR=1.06, 95%CI=1.0108-1.114, p= 0.022). This association was not observed in White men (p>0.05). Hence, we thought to further investigate the effect of obesity on the aforementioned SNPs (Table 2) and prostate cancer risk in Black and White men. The results are shown in the Table 3. The excessive prostate cancer risk associated with the rs7501939 SNP in the *HNF1B* appears to selectively affect obese Black men. Interestingly, no statistically significant associations were observed with either the BMI, or the SNPs and BMI and prostate cancer in White men (p>0.05).

Table 3. Association of the significant SNPs with obesity and prostate cancer risk in Black men.

SNP	Non-obese Black men: BMI<30	Obese Black men: BMI≥30	All Black men
Rs7501939 <i>HNF1B</i>	1.09 (0.65-1.83); 0.763	2.14 (1.2-3.8); 0.0103	1.56 (1.08-2.27); 0.0193
Rs4430796 <i>HNF1B</i>	0.82 (0.37-1.81); 0.62	0.53 (0.25-1.14); 0.10	0.57 (0.34-0.97); 0.0383

## Discussion

Results of our pilot study suggest that germline genetic variation in the metabolism-related genes, particularly *HNF1B*, affects prostate cancer risk in Black and White men. We also show that the risk estimates seem to differ by race and by obesity status. *HNF1B* encodes a member of a transcription factor superfamily, and as such modulates plethora of key processes such as proliferation.

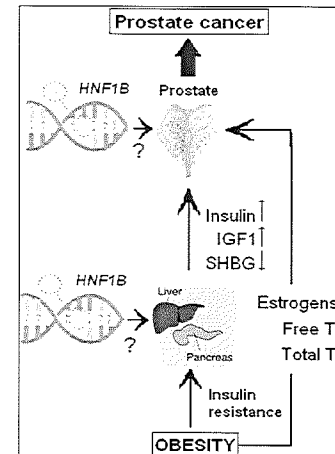


Figure 2. General schematic showing possible interactions between obesity, prostate cancer and *HNF1B*.

Genetic alterations in the *HNF1B* were linked to diabetes, urinary tract disorders, kidney malformations and pancreatic islet development defect. Different *HNF1B* isoforms were reported being expressed in cancerous versus normal prostate tissue, thus providing functional evidence for a role of this gene in prostate cancer development. Although the functional mechanisms of the *HNF1B* in prostate cancer development remain elusive, we propose the following general schematic of its action (Figure 2). We are planning the functional study to test and narrow this hypothesis.

## Conclusions

Our results suggest that germline genetic variation in the *HNF1B* is likely to differentially contribute to prostate cancer risk in Black men by obesity status. Future studies involving a large proportion of Black men are needed to elucidate the potential link between prostate cancer, obesity, diabetes and *HNF1B* genetic variation. In addition, functional tumor biology studies are warranted to elucidate the functional effects of the *HNF1B* on prostate cancer.

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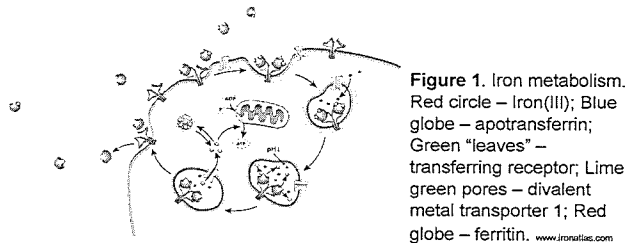
# Cellular Transport Gene Variants Are Associated With Susceptibility to Epithelial Ovarian Cancer and Histopathologic Subtypes

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## Background

- Epithelial ovarian carcinoma (EOC) is the most common gynecological malignancy in the United States (US).
- ~ 60% of cases are diagnosed at later stages (III-IV) with a 5 year overall survival of less than 20%.
- Disruptions in the cellular transport processes, such as iron transport (Figure 1), has been associated with increased risk of a number of cancers.
- Despite the growing evidence of association between the cellular transport processes and cancer risk, the association of germline genetic variation in the cellular transport genes and EOC risk has not been studied.
- We hypothesized that single nucleotide polymorphisms (SNPs) in the cellular transport genes are associated with EOC risk and histopathology.



**Figure 1.** Iron metabolism. Red circle – Iron(III); Blue globe – apotransferrin; Green “leaves” – transferrin receptor; Lime green pores – divalent metal transporter 1; Red globe – ferritin. www.ironlab.com

## Methods

- 305 SNPs from 120 transmembrane transport genes were genotyped in 14,496 cases and 23,191 control women of European ancestry from 43 studies in the Ovarian Cancer Association Consortium (OCAC).
- Genotyping was conducted using a custom Illumina iSelect designed for the Collaborative Oncological Gene-Environment Study (COGS).
- Both invasive cancers combined and the four main histological subtypes (serous [n=8,368], endometrioid [n=2,067], clear cell [n=1,024] and mucinous [n=944]) were analyzed, SNP analyses were conducted using unconditional logistic regression under a log-additive model.
- All analyses were adjusted for study site and population substructure.

## Results

- Characteristics of study participants are shown in Table 1. The mean age at diagnosis for women of European ancestry was 55 (std=11) years.
- The proportion of serous EOC (58%) was higher (15% endometrioid, 7% mucinous and 7% clear cell).
- Cases were older, had higher BMIs and were less likely to use hormonal contraceptives.
- Eighty-four SNPs remained statistically significantly associated with EOC risk (Table 2).

**Table 1.** Characteristics of study participants

Characteristic	Controls (N=23,191)		Cases (N=14,496)		P
	n	%	n	%	
Age (years, mean, std)	55.64	11.90	58.13	11.30	<0.0001
<40	2027	8.74	748	5.16	<0.0001
40-49	4771	20.57	2544	17.55	
50-59	7403	31.92	4537	31.30	
60-69	6098	26.29	4324	29.83	
≥70	2892	12.47	2343	16.16	
Age at menarche (years, mean, std)	12.88	1.71	12.83	1.59	.045
<12	3128	19.31	1856	19.22	0.08
12	3602	22.24	2257	23.37	
13	4357	26.90	2621	27.14	
≥14	5112	31.56	2923	30.27	
Body mass index (kg/m <sup>2</sup> )					0.0006
<25	3834	48.15	2528	45.10	
25-29	2332	29.29	1681	29.99	
≥30	1797	22.57	1396	24.91	
Oral contraceptive use					<0.0001
No	6136	37.49	4203	43.68	
Yes	10230	62.51	5419	56.32	
Histological subtypes					
Serous			8368	57.61	
Endometrioid			2067	14.23	
Clear Cell			1024	7.05	
Mucinous			944	6.50	
Other*			1643	11.31	

\* Includes mixed cell, other specified epithelial, undifferentiated and unknown histology

## Discussion

- Our results suggest involvement of the iron, copper, zinc, thyroid and glucuronosyltransferase transport in the EOC etiology.
- Our pilot analysis suggests that there are racial/ethnic differences in the EOC etiology across histopathologic subtypes.
- Hephaest (HEPH) showed the most significant association for all invasive EOC combined (OR=0.85; P=2.55\*10<sup>-4</sup>), as well as for Serous subtype (OR=0.81; P=1.99\*10<sup>-4</sup>) in European women. HEPH is a transmembrane copper-dependent ferroxidase, serving as a “gatekeeper” of iron available to the body.

- UDP glucuronosyltransferase 1 family, polypeptide A cluster (UGT1A) showed the most significant association for Endometrioid subtype (OR=0.82; P=6.8\*10<sup>-4</sup>) in European women. UGT1As are localized to endoplasmic reticulum and catalyze glucuronidation which results in an increased polarity of the substrate that facilitates its excretion.

- Calpain 13 (CAPN13) showed the most significant association for Clear Cell subtype (OR=0.86; P=0.014). CAPN13 is a calcium-dependent cysteine protease, participating in processes such as cell mobility and cell cycle.

**Table 1.** The top significant SNPs by EOC histologic subtype. INV: invasive; LMP: low malignant potential; Ser: serous; CC: clear cell; End: endometrioid; Muc: Mucinous.

	INV	LMP	Ser	CC	End	Muc	Asian (INV)	African (INV)
HEPH rs17216603	0.85 (0.77-0.93); 2.55*10 <sup>-4</sup>	0.78 (0.63-0.97); 0.0206	0.81 (0.73-0.91); 1.99*10 <sup>-4</sup>	0.77 (0.58-1.02); 0.07	0.9 (0.74-1.08); 0.2596	0.92 (0.7-1.21); 0.5955	1.46 (1.15-1.83); 0.0019	0.76 (0.15-3.86); 0.74
SLC39A11 rs9908917	0.95 (0.92-0.99); 0.014	0.85 (0.77-0.93); 3.5*10 <sup>-4</sup>	0.94 (0.9-0.99); 0.01012	1.01 (0.91-1.13); 0.82	0.94 (0.87-1.02); 0.16	0.98 (0.87-1.1); 0.7	1.25 (1.01-1.56); 0.049	1.23 (0.85-1.77); 0.27
SLC39A11 rs1522846	0.85 (0.81-0.89); 0.0026	1.00 (0.92-1.09); 0.92	0.96 (0.91-0.99); 0.0145	0.95 (0.85-1.06); 0.34	0.92 (0.86-1); 0.0465	0.95 (0.85-1.06); 0.36	1.34 (0.83-1.21); 0.008	0.81 (0.52-1.27); 0.36
SLC39A11 rs9913553	0.97 (0.93-1); 0.089	1.01 (0.94-1.1); 0.7	0.95 (0.91-0.99); 0.0133	1.05 (0.94-1.16); 0.38	0.95 (0.88-1.03); 0.23	1.01 (0.91-1.13); 0.84	0.83 (0.66-1.05); 0.12	1.06 (0.31-1.17); 0.13
SLC39A11 rs925846	1 (0.97-1.03); 0.01	1.03 (0.95-1.11); 0.46	1.02 (0.98-1.06); 0.37	0.88 (0.8-0.97); 0.0083	0.98 (0.88-1.09); 0.73	0.98 (0.88-1.09); 0.9	0.95 (0.41-2.22); 0.9	0.82 (0.63-1.35); 0.68
SLC39A11 rs9905659	1.01 (0.97-1.05); 0.56	1.05 (0.96-1.15); 0.29	1.03 (0.98-1.08); 0.27	0.88 (0.79-1); 0.045	0.98 (0.9-1.06); 0.61	1.01 (0.9-1.14); 0.53	1.06 (0.84-1.36); 0.61	1.54 (1.0-2.35); 0.047
SLC39A11 rs9913553	0.99 (0.94-1.04); 0.74	1.04 (0.93-1.17); 0.47	0.98 (0.92-1.04); 0.54	0.98 (0.84-1.13); 0.76	0.95 (0.86-1.06); 0.34	1.16 (1-1.34); 0.048	0.97 (0.78-1.22); 0.82	1.24 (0.87-1.75); 0.23
SERPINA7 rs1604455	1.05 (1-1.1); 0.04187	1.14 (1.03-1.27); 0.016	1.06 (1-1.12); 0.045	1.21 (1.06-1.39); 0.0042	1.08 (0.96-1.17); 0.28	0.85 (0.73-1); 0.045	0.81 (0.65-1.00); 0.0503	1.11 (0.75-1.65); 0.602
SLC25A45 rs681320	0.97 (0.94-1); 0.029	1.04 (0.97-1.12); 0.27	1 (0.96-1.04); 0.99	0.98 (0.89-1.07); 0.59	0.91 (0.85-0.97); 0.0035	0.89 (0.81-0.97); 0.012	0.78 (0.63-0.98); 0.033	0.78 (0.5-1.26); 0.41
UGT1A rs11562251	0.85 (0.81-0.9); 0.0502	1.08 (0.96-1.2); 0.21	0.95 (0.89-1.01); 0.074	1.03 (0.89-1.18); 0.73	0.82 (0.74-0.92); 5.8*10 <sup>-4</sup>	1.06 (0.91-1.22); 0.49	0.73 (0.5-1.06); 0.102	1.22 (0.85-1.75); 0.28
CAPN13 rs1562766	0.99 (0.96-1.02); 0.49	0.97 (0.9-1.04); 0.45	1.01 (0.97-1.04); 0.79	0.86 (0.79-0.95); 0.0014	0.99 (0.93-1.05); 0.69	1.02 (0.93-1.12); 0.71	1.08 (0.82-1.43); 0.56	1.14 (0.73-1.79); 0.57
CAPN13 rs4952165	0.98 (0.95-1.01); 0.15	0.94 (0.87-1.01); 0.086	0.98 (0.94-1.02); 0.27	0.88 (0.8-0.97); 0.0102	1.02 (0.95-1.09); 0.67	0.97 (0.88-1.07); 0.53	0.85 (0.67-1.08); 0.19	0.78 (0.51-1.19); 0.25
MGST1 rs6488840	0.96 (0.93-1); 0.048	1.0 (0.92-1.09); 0.902	0.96 (0.91-1); 0.042	0.96 (0.86-1.07); 0.41	1 (0.92-1.08); 0.95	0.98 (0.86-1.08); 0.48	0.54 (0.25-1.16); 0.12	0.66 (0.37-0.82); 0.0035

- Solute carrier family 25, member 45 (SLC25A45) showed the most significant association for Mucinous subtype (OR=0.89; P=0.012). SLC25A45 catalyzes the transport of solutes (ADP/ATP, amino acids, long chain fatty acids) across the inner mitochondrial membrane.

- In Asian women, HEPH showed the most significant association (OR=1.45; P=0.0019). In African women, Mitochondrial glutathione S-transferase 1 (MGST1) showed the most significant association (OR=0.55; P=0.0035).

## Conclusions

Our results, generated on a large cohort of women, suggest specific associations between inherited cellular transport gene variants, particularly metal transporters, and susceptibility to different EOC histopathologic subtypes.

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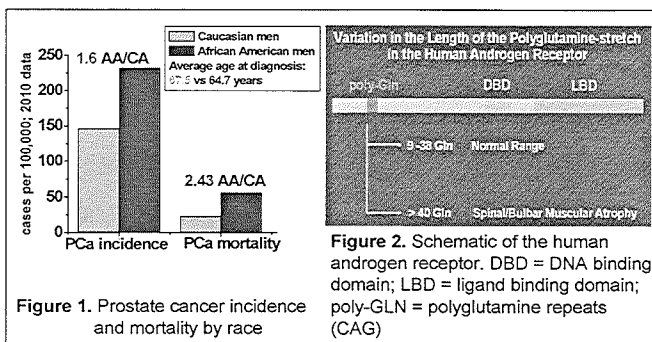
# Androgen Receptor CAG Repeat Length and Association with Prostate Cancer Risk in Obese and Non-Obese African American men

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## Background

In the United States, African American/ Black men (AAM) are 60% more likely to be diagnosed with, and 140% more likely to die from, prostate cancer compared to their European American/ White (EAM) counterparts (Figure 1). Despite significant prostate cancer burden in this exceptionally high risk population, the reasons for the disparity are not well understood; however, existing evidence supports a role for a genetic component. The trinucleotide (CAG)(n) repeat polymorphism in exon 1 of the androgen receptor (AR) gene regulates AR activity, with longer alleles conferring reduced activity (Figure 2). Significant interethnic variations in the allele length frequencies of the AR (CAG)(n) polymorphism repeat have been reported. We investigated the association between the AR CAG(n) repeat length and prostate cancer risk in AAM and EAM. We further explored if this association varied by body mass index (BMI) in the two races.



## Methods

Study participants were recruited from various hospitals in Tampa Bay. Eligible subjects self-classified as either AAM or EAM and were 30-80 years old. Cases had biopsy-confirmed prostate cancer and had no prior treatment at the time of enrollment. Controls were defined as men having no evidence of prostate cancer on biopsy and/or low PSA levels. All men signed an informed consent form and provided a blood or saliva sample for DNA. We tested DNA samples from 259 (136 cases and 123 controls) AA and 269 (147 cases and 142 controls) NHW men, matched by age, Gleason score and PSA. The CAG repeat region was PCR amplified, and the PCR fragments were analyzed using the Beckman Coulter CEQ8000 DNA sequencer to establish the repeat length.

## Statistical analyses

Descriptive statistics was performed for discrete and continuous variables. Associations between repeats and prostate cancer risk were evaluated using unconditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (95%CI) for each race. Repeats were categorized using the median (22 repeats) as a cutpoint. Significance was defined as the p-value <0.05.

## Results

DNA samples from 259 (136 cases and 123 controls) AAM and 269 (147 cases and 142 controls) EAM were analyzed, yielding a total sample size of 528 men. EAM were matched to AAM by case status, age, and Gleason score. Select characteristics of the study participants by case/control status are shown in the Table 1. For both ethnic groups, men were likely to be between 50 to 64 years of age and between 68 and 72 inches tall.

Table 1. Select characteristics of the study participants by case/control status

	AAM: 259 total		EAM: 269 total	
	# Cases (%)	# Controls (%)	# Cases (%)	# Controls (%)
Age				
<50	11 (8)	30 (24)	12 (8)	19 (16)
50-64	74 (54)	73 (59)	80 (54)	85 (70)
≥65	51 (38)	17 (14)	55 (38)	18 (14)
missing	NA	3 (3)	NA	NA
BMI				
<24.9 normal weight	9 (7)	26 (21)	25 (17)	28 (23)
25.0-29.9 overweight	47 (34.5)	48 (37)	70 (48)	47 (38)
≥30.0 obese	71 (52)	51 (42)	48 (33)	46 (38)
missing	9 (6.5)	NA	4 (2)	1 (1)
Height				
≤67 in	30 (22)	21 (17)	26 (17.5)	22 (18)
68-72 in	65 (48)	74 (60)	98 (66.5)	79 (65)
≥73 in	32 (23.5)	28 (23)	19 (13)	21 (17)
missing	9 (6.5)	NA	4 (3)	NA

Within ethnic groups, AAM cases were more likely to be obese than AAM controls. In contrast, EAM controls were more likely to be obese than EAM cases. AAM were more likely to be obese and taller than EAM. However, these differences were not statistically significant (p>0.05).

Results for the CAG repeat analysis and prostate cancer status are shown in the Table 2. No statistically significant associations were noted when the repeat length was treated as a continuous variable. However, when a binary cutpoint was used (a median repeat length in our study, CAG=22), AAM with longer repeats (≥22) demonstrated increased prostate cancer risk. Interestingly, the increased risk was only evident in obese (BMI≥30), but not in the non-obese (BMI<30) AAM. No associations were noted in EAM regardless of the BMI.

Table 2. AR CAG-repeat length and prostate cancer status. "+" denotes a case; "-" denotes a control.

	AAM				EAM				Combined			
	+	-	OR (95%CI)	P	+	-	OR (95%CI)	P	+	-	OR (95%CI)	P
Continuous	147	122	1.00 (0.94-1.07)	0.94	136	123	1.06 (1.00-1.17)	0.06	283	245	1.04 (0.99-1.09)	0.17
Binary all												
<22	40	49	Reference		66	66	Reference		106	115	Reference	
≥22	88	63	1.81 (1.05-3.13)	0.034	53	50	0.96 (0.56-1.65)	0.87	136	116	1.37 (0.90-1.92)	0.15
Non-obese												
<22	30	31	Reference		28	38	Reference		58	69	Reference	
≥22	54	39	1.43 (0.73-2.77)	0.30	19	33	0.80 (0.35-1.83)	0.60	73	72	1.22 (0.73-2.01)	0.45
Obese												
<22	9	18	Reference		34	28	Reference		43	46	Reference	
≥22	29	23	3.12 (1.12-8.73)	0.03	27	20	1.11 (0.51-2.41)	0.79	56	43	1.45 (0.18-2.60)	0.21

We have analyzed the CAG repeat distribution using other categories commonly used in other studies (<19; 12-25; and ≥26 repeats). None of the analyses were significant using these categories (p>0.05).

## Discussion

Obesity (BMI≥30) alters individual's hormonal profile; specifically, estrogens are increased, and androgens are decreased in obese compared to normal weight men. While the majority of early prostate cancers rely on androgens, depletion of androgens (castration) eventually leads to an androgen independent malignancy. Our provocative, very preliminary findings may indicate that obese AAM with altered androgen/estrogen profile (androgen ↓, estrogen ↑) and reduced activity AR receptors may be more susceptible to develop prostate cancers (Table 3). Further, we are tempted to hypothesize that obese AAM with longer CAG repeats are prone to develop an androgen-independent malignancy that may be more aggressive and/or resistant to conventional treatments. Additional research is needed to test this hypothesis as well as establish possible reasons why this effect is observed in AAM but not in EAM.

Table 3. Androgen receptor (AR) activity, androgen/estrogen balances and prostate cancer in AAM

AR activity	Androgen/estrogen	
	Normal/normal (BMI<30)	Low/High (BMI≥30)
Normal (CAG<22)	Conventional slow-growing prostate cancer	? Still conventional slow-growing cancer ?
Reduced (CAG≥22)	? Still conventional slow-growing prostate cancer ?	Androgen-independent, aggressive cancer

## Conclusions

Our results suggest that prostate cancer risk may be increased in AAM with longer AR CAG repeats (≥22), especially obese (BMI≥30) AAM. The data provide evidence for ethnic differences in androgenic pathway activity and androgen sensitivity which may contribute to prostate cancer disparity.

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App. 10

**Variation in *HNF1B* and obesity may influence prostate cancer risk in African American men: a pilot study**

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## Abstract

**Background.** Prostate cancer (PCa) racial disparity is multifactorial, involving biological, as well as socio-cultural and lifestyle determinants. We investigated the association between selected potentially functional polymorphisms and PCa risk in African American/Black (AAM) and Non-Hispanic White (EAM) men. We further explored if these associations varied by the body mass index (BMI) and height in each race.

**Methods.** Age-matched DNA samples from 259 (136 cases and 123 controls) AAM and 269 (147 cases and 142 controls) EAM, were genotyped for 10 candidate single nucleotide polymorphisms (SNPs) in 7 genes using the TaqMan allelic differentiation analysis. The dominant, recessive and additive age-adjusted unconditional logistic regression models were fitted for all men combined, for each race and stratified by BMI and height within each race.

**Results.** Three SNPs showed statistically significant associations with PCa risk. In AAM: *HNF1B* rs7501939 (OR=2.42, 95%CI=1.31-4.47, P=0.0046) and rs4430796 (OR=0.57, 95%CI=0.34-0.97, P=0.0383), which were both previously associated with the risk for type II diabetes. In EAM: *CTBP2* rs4962416 (OR=1.52, 95%CI=1.02-2.26, P=0.0384). In addition, high BMI in AAM (OR=1.06, 95%CI=1.01-1.114, P=0.022), and height in EAM (OR=0.92, 95%CI=0.85-0.99, P=0.0434) showed significant associations. Interestingly, *HNF1B* rs7501939 was associated with PCa exclusively in obese AAM (OR=2.14, 95%CI=1.2-3.8, P=0.0103).

**Conclusion.** Our results suggest that variation in the *HNF1B* may influence PCa risk in obese AAM.

## Introduction

Prostate cancer (PCa) remains the most common type of solid malignancy, and the second-leading cause of all cancer death in U.S. men [1]. However, the burden of PCa is not the same across all racial and ethnic groups, as African American / Black men (AAM) consistently demonstrate 1.6 times higher incidence, and 2-3 times higher mortality rates of PCa, compared to their Non-Hispanic White (EAM) counterparts [2]. In addition, AAM are more likely to be diagnosed at an earlier age, have more aggressive tumors and higher recurrence rates following definite treatments [2-3]. The etiology of racial disparity in PCa is thought to be multifactorial, involving biological, as well as socio-cultural and lifestyle determinants [4]. Although genome-wide association studies (GWAS) have identified more than a dozen PCa risk loci [5], elucidating the biological basis for these associations is challenging [6]. The post-GWAS studies are increasingly suggestive of the interaction between genetic variants and environmental risk factors [7] for which our understanding is still largely inadequate [8].

Established risk factors for PCa are increasing age, race and a family history of the disease [2-4]. Obesity (which affects 35% of all U.S. adults [9] and is more prevalent in African American population [10]) is linked to plethora of diseases including cardio-vascular problems, type II diabetes, gallbladder disease, osteoarthritis [11] and an array of human cancers such as breast, uterine, and pancreas [11-13]. Furthermore, obesity alters the individual's biochemical and hormonal profile [14], which may facilitate cancer growth [15]. However, obesity has been inconsistently associated with PCa risk [16], and the inconsistency may be due to an interaction with genetic variants [17-18]. In an attempt to elucidate the connection between PCa health disparity, genetic variation and obesity, we hypothesized that genetic variation differentially alters the PCa risk in obese and non-obese AAM and EAM. Given the extremely high burden of PCa and staggering rates of obesity, elucidating the links between the individual's genetic variation, race, PCa risk and obesity is likely to have a major positive impact on the public health



of the US population. Thus, our hypothesis-generating study may open new venues for tackling the PCa disparity from a new perspective.

## **Methods**

### **Study participants**

Study participants were recruited from various clinics in the Tampa Bay area in Florida, including the Moffitt Cancer Center, Tampa Bay Radiation Oncology centers, Moffitt Cancer Center affiliated-Lifetime Cancer Screening & Prevention Center, James A. Haley Veteran Affairs (VA), and the 30<sup>th</sup> Street Medical Associates (a community clinic). All recruitment protocols were approved by the University of South Florida Institutional Review Board (IRB), while the VA protocol was approved by the VA IRB. The study population comprised of AAM and EAM aged 30-85 years and enrolled between 2006 and 2012. The cases and controls were recruited during the initial PCa screening of all consecutive, unselected patients. Cases were histologically -confirmed PCa patients and controls were men with low PSA and/or no evidence of PCa on biopsy. The AAM or EAM ancestry was self-reported. Men were excluded if they did not self-identify as either AAM or EAM, were outside of the 30-85 year old range, were in poor physical or mental health, were diagnosed with other cancers (excluding non-melanoma skin cancer), or did not speak English well enough to read and understand the informed consent. The response rates in all studies were high, at or above 90%.

### **Single nucleotide polymorphism (SNP) selection and genotyping**

Literature search using Pubmed and Google scholar databases was performed to identify potential SNPs of interest. The following criteria were set to guide the SNP search (all inclusive): 1. Confer increased PCa risk in AAM; 2. Confer increased PCa risk in EAM; 3. Demonstrate potential for functional significance (that is, located in or close to a gene with a known function); 4. Reported Minor allele frequency (MAF)  $\geq 15\%$  in AAM and EAM. Based on these criteria, 10 SNPs in the 7 genes were selected: rs4430796; rs7501939; rs1859962 in *HNF1B*; rs10993994 in *MSMB*; rs822396 in *ADIPOQ*; rs4263970; rs4612601 in *EPHB2*;

rs4962416 in *CTBP2*; rs627839 in *RNASEL*; rs2070874 in *IL4*. DNA was extracted from blood or buccal cell samples using commercially available extraction kits and TaqMan genotyping was conducted at the Moffitt Cancer Center on the DNA samples from 259 (136 cases and 123 controls) AAM and 269 (147 cases and 142 controls) EAM, matched on age at diagnosis.

### **Statistical Analyses**

Descriptive statistics were used to summarize participants' demographic and clinical characteristics. Genotypes among participants were used to estimate allele frequencies and departure from Hardy-Weinberg equilibrium (HWE) was assessed among control subjects using Chi-squared test. The association between each SNP and PCa risk was estimated with odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression adjusted for age at diagnosis. Three inheritance genetic models (log-additive, dominant and recessive) were tested for each SNP and the model with the minimum *P*-value was considered as the best fitting model. Separate analyses were performed for each race and all men combined. We conducted exploratory subgroup analyses for different strata based on BMI and height within each race. Statistical tests were two-sided with an alpha level <0.05 considered statistically significant. All statistical analyses were performed with SAS/Genetics version 9.2 (SAS Institute, NC).

### **Results**

Selected characteristics of the study participants by case/control status are shown in the Table 1. For both ethnic groups, men were likely to be between 50 to 64 years of age and between 68 and 72 inches tall. AAM were more likely to be obese than EAM (47% and 35.5% obese men in each group, respectively). AAM were also more likely to be taller. Within each ethnic group, cases were more likely to be older, although there was no significant difference in the age of cases and controls between races. In addition, AAM cases were more likely to be obese than AAM controls. In contrast, EAM controls were more likely to be obese than EAM cases.

The results for association analyses between SNP and risk are shown in the Table 2. The MAF of the SNPs ranged from 0.15-0.49 and none of the SNPs deviated from HWE (all  $P > 0.05$ ). In AAM, we observed an increased PCa risk at *HNF1B* rs7501939 (Recessive model: OR=2.42, 95%CI=1.31-4.47,  $P=0.0046$ ; Additive model: OR=1.56, 95%CI=1.08-2.27,  $P=0.0193$ ) and a decreased PCa risk at *HNF1B* rs4430796 (Dominant model: OR=0.57, 95%CI=0.34-0.97,  $P=0.0383$ ; Additive model: OR=0.67, 95%CI=0.46-0.99,  $P=0.0431$ ). These SNPs were not significantly associated with PCa risk in EAM. In EAM, we observed an increased PCa risk at the *CTBP2* rs4962416 (Dominant model: OR=1.69, 95%CI=1.02-2.80,  $P=0.0415$ ; Additive model: OR=1.52, 95%CI=1.02-2.26,  $P=0.0384$ ). This association was not confirmed in AAM.

Age-adjusted association between select anthropometric variables and PCa are shown in the Table 3. We observed an increased PCa risk in obese, compared to non-obese AAM (OR=1.06, 95%CI=1.01-1.11,  $P=0.022$ ), and decreased PCa risk in the tallest group of EAM compared to all other EAM (OR=0.92, CI=0.85-0.99,  $P=0.0434$ ). Since our results indicated that BMI might be positively associated with PCa in AAM, we decided to examine the SNP-associations stratified by obesity for SNPs that were significant for AAM: rs7501939 and rs4430796.

Results for SNP-associations stratified by obesity in AAM are shown in Table 4. Interestingly, when stratified by obesity status, rs7501939 at *HNF1B* only increased PCa risk in obese AAM (OR=2.14, 95%CI=1.2-3.8,  $P=0.01$ ), but not in the non-obese AAM ( $P=0.76$ ), or EAM of any BMI ( $P=0.3$  in obese, and  $p=0.8$  in non-obese EAM). No differential association by obesity status was observed at rs4430796 in AAM ( $P=0.18$  in obese and  $P=0.51$  in the non-obese AAM). There were no significant associations with PCa risk at these SNP in EAM regardless of obesity status ( $p>0.05$ ).

Finally, we used the SNP Annotation and Proxy Search (SNAP) software (Broad Institute) to elucidate whether there are other SNPs in Linkage Disequilibrium (LD) with our significant SNPs. The results are shown in the Table 5. There are no SNPs in the LD ( $r^2 \geq 0.8$ )

with either of the *HNF1B* SNPs significant in AAM. While there are several SNPs in weak LD ( $r^2 \geq 0.5$ ) with rs7501939, they were not found to be associated with PCa or any other disease. In EAM, while a number of SNPs are reported being in LD with the CTBP2 rs4962416, only one of those SNPs (rs12769019) was linked to a marginally increased PCa risk in EAM (OR=1.1, 95% CI, 0.99-1.25) [19].

Table 1. Select characteristics of the study participants by case/control status.

		AAM: 259 total		EAM: 269 total		Total sample combined	
		# Cases (%) 136 (52.5)	# Controls (%) 123 (47.5)	# Cases (%) 147 (54.6)	# Controls (%) 122 (45.4)	# Cases (%) 283 (53.6)	# Controls (%) 245 (46.4)
Age	<50	11 (8)	12 (8)	19 (16)	30 (24)	30 (12)	42 (16)
	50-64	74 (54)	80 (54)	85 (70)	73 (59)	159 (62)	153 (56.5)
	≥65	51 (38)	55 (38)	18 (14)	17 (14)	69 (26)	72 (26)
	missing	NA	NA	NA	3 (3)	NA	3 (1.5)
BMI	≤24.9 normal weight	9 (7)	25 (17)	28 (23)	26 (21)	37 (15)	51 (19)
	25.0-29.9 overweight	47 (34.5)	70 (48)	47 (38)	46 (37)	94 (36)	116 (42.5)
	≥30.0 obese	71 (52)	48 (33)	46 (38)	51 (42)	58.5 (45)	49.5 (37.5)
	missing	9 (6.5)	4 (2)	1 (1)	NA	10 (3.5)	4 (1)
Height	≤67	30 (22)	26 (17.5)	22 (18)	21 (17)	52 (20)	47 (17)
	68-72	65 (48)	98 (66.5)	79 (65)	74 (60)	144 (56.5)	172 (63)
	≥73	32 (23.5)	19 (13)	21 (17)	28 (23)	53 (20)	47 (18)
	missing	9 (6.5)	4 (3)	NA	NA	9 (3)	4 (1.5)

Table 2. Age-adjusted odds ratios (OR) and 95% confidence intervals (95%CI) for PCa in AAM and EAM and combined.

Rs# and gene; minor allele and its frequency	Dominant Model OR (CI); p-value	Recessive Model OR(CI); p-value	Additive Model OR(CI); p-value
rs4612601; EPHB2 AA: 0.49(G) EA: 0.45(A)	AAM: 1.09 (0.63-1.9); 0.749 EAM: 1.18 (0.67-2.10); 0.564 All: 1.13 (0.76-1.69); 0.537	0.86 (0.46-1.61); 0.638 0.9 (0.52-1.56); 0.709 0.89 (0.59-1.34); 0.565	0.99 (0.69-1.41); 0.951 1.02 (0.73-1.43); 0.912 1.00 (0.79-1.28); 0.969
rs4263970; EPHB2 AA: 0.47(T) EA: 0.45(C)	AAM: 0.98 (0.57-1.68); 0.932 EAM: 1.08 (0.61-1.9); 0.795 All: 1.02 (0.69-1.5); 0.918	0.88 (0.44-1.75); 0.711 0.76 (0.44-1.33); 0.334 0.81 (0.53-1.25); 0.343	0.95 (0.66-1.38); 0.797 0.93 (0.66-1.30); 0.665 0.94 (0.73-1.21); 0.630
rs822396; ADIPOQ AA: 0.2(G) EA: 0.15(G)	AAM: 0.89 (0.52-1.52); 0.671 EAM: 0.75 (0.44-1.27); 0.288 All: 0.82 (0.56-1.19); 0.285	1.64 (0.37-7.21); 0.511 1.1 (0.29-4.09); 0.094 1.29 (0.49-3.48); 0.601	0.96 (0.6-1.54); 0.878 0.82 (0.53-1.29); 0.392 0.88 (0.64-1.22); 0.456

rs10993994; MSMB	AAM: 0.82 (0.48-1.4); 0.472	1.04 (0.51-2.10); 0.919	0.92 (0.63-1.33); 0.657
AA: 0.2 (C)	EAM: 1.04 (0.54-2.00); 0.899	0.61 (0.36-1.05); 0.077	0.81 (0.56-1.17); 0.258
EA: 0.34(T)	All: 0.9 (0.6-1.35); 0.611	0.75 (0.49-1.14); 0.177	0.87 (0.67-1.11); 0.261
rs1859962; HNF1B	AAM: 1.03 (0.61-1.74); 0.905	1.23 (0.54-2.81); 0.626	1.06 (0.72-1.56); 0.752
AA: 0.49(G)	EAM: 1.38 (0.77-2.45); 0.278	1.00 (0.57-1.76); 0.997	1.13 (0.79-1.6); 0.508
EA: 0.2(G)	All: 1.16 (0.8-1.69); 0.431	1.06 (0.67-1.69); 0.790	1.09 (0.85-1.4); 0.503
rs7501939; HNF1B	AAM: 1.34 (0.73-2.47); 0.343	<b>2.42 (1.31-4.47); 0.0046</b>	<b>1.56 (1.08-2.27); 0.0193</b>
AA: 0.48(C)	EAM: 0.82 (0.5-1.35); 0.439	1.13 (0.54-2.38); 0.742	0.93 (0.65-1.33); 0.692
EA: 0.49(T)	All: 0.99 (0.68-1.45); 0.981	<b>1.76 (1.11-2.79); 0.0167</b>	1.18 (0.92-1.52); 0.187
rs4430796; HNF1B	AAM: <b>0.57 (0.34-0.97); 0.0383</b>	0.64 (0.29-1.42); 0.272	<b>0.67 (0.46-0.99); 0.0431</b>
AA: 0.34(A)	EAM: 1.27 (0.7-2.3); 0.439	0.98 (0.57-1.68); 0.946	1.07 (0.76-1.51); 0.689
EA: 0.47(A)	All: 0.81 (0.55-1.18); 0.273	0.86 (0.59-1.33); 0.500	0.87 (0.69-1.12); 0.279
rs2070874; IL4	AAM: 0.94 (0.54-1.64); 0.824	1.45 (0.7-3.0); 0.314	1.08 (0.73-1.59); 0.700
AA: 0.45(T)	EAM: 0.65 (0.38-1.11); 0.115	0.92 (0.22-3.93); 0.916	0.72 (0.45-1.14); 0.161
EA: 0.2(T)	All: 0.82 (0.57-1.17); 0.275	1.32 (0.7-2.49); 0.386	0.94 (0.71-1.24); 0.649
rs627839; RNASEL	AAM: 1.2 (0.7-2.06); 0.511	1.63 (0.78-3.42); 0.192	1.24 (0.85-1.83); 0.252
AA: 0.46(T)	EAM: 0.86 (0.5-1.47); 0.571	0.99 (0.55-1.8); 0.986	0.94 (0.67-1.32); 0.710
EA: 0.45(T)	All: 1.01 (0.69-1.48); 0.952	1.21 (0.76-1.91); 0.427	1.06 (0.83-1.37); 0.632
rs4962416; CTBP2	AAM: 0.9 (0.5-1.59); 0.709	5.65 (0.62-51.7); 0.125	1.05 (0.63-1.74); 0.861
AA: 0.23(C)	<b>EAM: 1.69 (1.02-2.8); 0.0415</b>	1.72 (0.68-4.31); 0.251	<b>1.52 (1.02-2.26); 0.0384</b>
EA: 0.23(C)	All: 1.28 (0.88-1.85); 0.198	2.12 (0.92-4.9); 0.0784	1.31 (0.97-1.78); 0.083

Table 3. Age-adjusted association between select anthropometric variables and PCa in men stratifying by race and combined. **Bold** denotes statistically significant associations.

	AAM	EAM	All
	OR (95% CI); p-value	OR (95% CI); p-value	OR (95% CI); p-value
Height (inches)	0.98 (0.9-1.05); 0.54	<b>0.92(0.85-0.99);0.0434</b>	0.95(0.89-1.01); 0.0549
BMI (kg/m2)	<b>1.06(1.01-1.11); 0.022</b>	0.98(0.93-1.023); 0.33	1.02 (0.98-1.05); 0.374
<30 (Non-obese)	1.0	1.0	1.0
≥30 (Obese)	<b>2.07 (1.21-3.55); 0.008</b>	0.90 (0.54-1.51); 0.69	1.32 (0.97-1.77); 0.09

Table 4. rs7501939 and rs4430796 as PCa risk factors in AAM stratified by BMI. Statistically significant associations are shown in **bold**.

	Non-obese AAM	Obese AAM	All AAM
rs7501939	1.09 (0.65-1.83); 0.75	<b>2.13 (1.20-3.81); 0.01</b>	<b>1.56 (1.08-2.27); P=0.019</b>
rs4430796	0.84 (0.49-1.43); 0.52	0.67 (0.37-1.20); 0.18	<b>0.67 (0.46-0.99); 0.043</b>

Table 5. SNPs reported being in LD regions with our SNPs of interest.

SNP of interest	$r^2 \geq 0.8$	$r^2 \geq 0.5$	Disease and comments
rs7501939 in AAM	none	rs11657964 ( $r^2=0.61$ ) rs8064454 ( $r^2=0.549$ )	None reported being associated with prostate cancer or any other disease.
rs4430796 in AAM	none	none	NA
rs4962416 in EAM	rs4962720 ( $r^2=1.00$ ) rs4962419 ( $r^2=1.00$ ) rs12771627 ( $r^2=1.00$ ) rs12769019 ( $r^2=0.92$ ) rs11598549 ( $r^2=0.92$ ) rs12782469 ( $r^2=0.83$ )	14 additional SNPs	rs12769019: slightly increased risk for prostate cancer in EAM (OR=1.1) [19];

## Discussion

We observe that the *HNF1B* SNPs (rs7501939 and rs4430796) identified in PCa GWAS [20-21] are associated with PCa risk in AAM, and that the *CTBP2* SNP rs4962416, also identified in PCa GWAS [20], is associated with PCa risk in EAM. Our novel finding is that the association of rs7501939 with PCa risk in AAM may be modified by obesity.

Gudmundsson et al [21] reported that *HNF1B* SNPs were associated with PCa in the Non-Hispanic White men (largely living in Europe) on the genome-wide significance level (rs7501939: OR=1.19,  $P=4.7 \times 10^{-9}$ ; rs4430796: OR=1.22,  $P=1.4 \times 10^{-11}$ ). The authors also reported that the same SNPs were inversely associated with the type II diabetes (rs7501939: OR=0.91,  $p=9.2 \times 10^{-7}$ ; rs4430796: OR=0.91,  $p=2.7 \times 10^{-7}$ ). Liu et al [22] conducted a meta-analysis of all PCa-related GWA studies combined, and stratified by ethnicity. The authors have found that in a combined analysis both *HNF1B* SNPs were significantly associated with PCa (rs7501939: OR=1.19,  $P<0.001$ ; rs4430796: OR=0.75,  $P<0.001$ ). However, neither of the two SNPs attained statistical significance when analysis was restricted to AAM ( $P>0.05$ ). Stevens et al [23] reported that the two *HNF1B* SNPs of interest were significantly inversely associated with PCa risk in their cohorts of EAM (rs7501939: OR=0.87, CI=0.80-0.94; rs4430796: OR=0.85, CI=0.79-0.92). However, unlike Gudmundsson et al [21], the authors did not observe a statistically significant association with diabetes. Sun et al [24] analyzed a diverse cohort of men (2139 EAM and 717 AAM) and reported associations with PCa risk and both SNPs in EAM (rs7501939: OR=1.31,  $P=1.8 \times 10^{-4}$ ; rs4430796: OR=1.28,  $P=5.1 \times 10^{-4}$ ). However, in an analysis restricted to AAM, only rs4430796 retained statistical significance (rs75001939:  $P>0.05$ ; rs4430796: OR=1.26,  $P=0.04$ ). The authors used reversed risk alleles (T for rs4430796 and G for rs75001939) which is opposite to our study and to other cited studies. Ahn et al. [25] reported that rs4430796 was not associated with risk for metastatic prostate cancer and recurrence. Although none of the aforementioned studies controlled for BMI, Lindstrom et al [26]

have reported that BMI had no modifying effects on the SNP-PCa associations in EAM. No similar analysis was performed in AAM.

While the aforementioned studies involved mostly EAM participants, Chang *et al.* [27] could not validate the association of either of the two *HNF1B* SNPs with PCa in their large cohort of 4,040 AAM PCa cases and 3,748 healthy AAM controls (rs7501939: OR=0.97, P=0.65; rs4430796: OR=1.08, P=0.053). Haiman *et al.* [28] reported no association in their large cohort of 3,621 AAM PCa cases and 3,652 controls (rs7501939: OR=1.03, P=0.44; rs4430796: OR=1.02, P=0.52). Hooker *et al.* [29] have attempted to replicate the PCa SNPs identified in the GWAS studies, in their cohort of 755 unrelated AAM. The authors reported that *HNF1B* rs7501939 was not significant (OR=0.94, P=0.20), and rs4430796 conferred increased PCa risk (OR=1.48, P=0.008).

As can be concluded from the referenced studies, there is significant discordance between the published studies on the effects of the two aforementioned *HNF1B* SNPs on PCa risk. While the authors presented high-quality studies with large sample sizes, the majority did not report controlling for the environmental confounders, including BMI. To our knowledge, we are the first group to report that the association of the *HNF1B* SNPs with PCa risk may be modified by the level of adiposity and racial/ethnic background. Importantly, BMI was associated with PCa risk in our sample of AAM independently of genetic variation (OR=1.06, P=0.022) (Table 3). Our hypothesis-generating data may be even more intriguing in light of the ongoing debate surrounding the relationship between obesity and PCa risk. At present, there appears to be a consensus that obese men experience slightly reduced overall PCa incidence, at the expense of an increase in aggressive, “clinically significant” disease. The overall decrease, therefore, is mainly due to reduction in the “clinically insignificant”, potentially indolent PCa [14]. While several hypotheses aimed to explain this observation have been proposed [30], neither has been widely accepted by scientific or medical communities. Additionally, because the data were obtained in EAM, the effect of obesity on PCa risk in AAM remains unknown. Our pilot

study helps to close this knowledge gap and serve as a basis for future sufficiently powered studies involving larger numbers of AA participants, as well as more extensive epidemiological data analysis.

Mutations in the *HNF1B* cause MODY5 (Maturity-Onset of Diabetes, Type 5) that may be accompanied by urinary tract disorders, including renal disease and/or undeveloped/malformed kidneys, and atrophic pancreas [31-32]. Some men with the *HNF1B* mutations have malformations in the reproductive tract including epididymal cysts, agenesis of the vas deferens, or infertility due to abnormal spermatozoa [33]. More recently, genetic variants in the *HNF1B* were implicated in the prostate [34-35] and endometrial [36-37] cancer risks. It was reported [38] that different *HNF1B* isoforms were expressed in prostate tumors versus normal prostate tissue, thus providing functional evidence for a potential role of this gene in PCa. However, the functional studies to examine whether *HNF1B* variants influence PCa risk and/or prognosis are lacking. *HNF1B* variants were implicated in a slightly reduced risk of type II diabetes mellitus in AAM and EAM [21]. Interestingly, PCa risk seems to be attenuated in men with diabetes [39], although the latter was only reported in people of EA descent and the biological basis for this association remains to be elucidated. At this time, the relationship between obesity, diabetes and PCa is poorly understood, and so is the contribution of the *HNF1B* variants to the risk. However, since obesity as a risk factor for breast cancer differs in AA and EA women [40-41], it is plausible that a similar effect may be observed with obesity and PCa in AAM and EAM. Additionally, our data suggests that *HNF1B* variants may be implicated in the risk. Future epidemiological, genetic and functional tumor biology studies are required to address this provocative hypothesis.

In EAM, our results replicate a previously reported finding that the *CTBP2* rs4962416 confers increased PCa risk: OR=1.69, p=0.0415 in our study vs. the overall GWAS data: OR=1.25, p=0.004 [22]. *CTBP2* encodes a transcriptional co-repressor that is activated under stress condition and can mediate stress-induced migration of tumor cells [42]. *CTBP2*



expression is detected in the prostate, and has been linked to decreased *PTEN* expression and activation of the phosphatidylinositol 3-kinase pathway [43] which may support or promote PCa growth. rs4962416 was not previously implicated in PCa risk in AAM [22] which is also in agreement with our data.

The main strength of our study is inclusion of equal proportions of age-matched AA and EA men with high rates of obese men in both populations, allowing us to tease out the interaction of obesity with genetic variants. Our results should be interpreted in light of limitations of a small sample size and inability to access relevant information such as diabetes history. However, given the increased PCa burden in AAM, alarming obesity rates, and limited number of studies that involve AAM, our results are novel, timely and as such, deserve dissemination.

In summary, our results suggest that germline genetic variation in *HNF1B* and *CTBP2* differentially contribute to PCa risk in men of different races and adiposities. Future sufficiently powered studies involving the large proportion of AAM are needed to elucidate the potential connection between the race, PCa, obesity, diabetes and *HNF1B*. In addition, functional tumor biology studies are warranted to elucidate the effects of obesity on PCa in carriers and non-carriers of different races and ethnicities.

### **Conflict of Interest**

All authors declare that they have no conflict of interest.

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## Authors' contributions

Conception and design: GC; NK; CP; JYP

Data collection and analysis: GC; EA; SD

Writing and editing of the manuscript: GC; EA; SD

Study supervision: JPS; NK

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**Incidence of colorectal cancer in *BRCA1* and *BRCA2* mutation carriers: Results from a follow-up study.**

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## ABSTRACT

**Background:** The *BRCA1* and *BRCA2* genes confer increased susceptibility to breast and ovarian cancer and to cancer types. There is controversy regarding the risk of colorectal cancer conferred by germline mutations in these two genes. We estimated the incidence of colorectal cancer in a cohort of female carriers of *BRCA1* and *BRCA2* mutation carriers from the six countries.

**Methods:** We followed 7,015 women with a *BRCA* mutation for new cases of colorectal cancer. Incidence rates in carriers were compared with population-specific incidence rates, and relative risks (SIRs) were estimated. The expected numbers of cancers were computed by multiplying person-years at risk by the appropriate age-, sex- and country-specific incidence rates from the six countries.

**Results:** Twenty-two incident colorectal cancer cases were observed among all mutation carriers, compared to 23.6 cases expected. The SIR for *BRCA1* carriers was 0.97 (95%CI, 0.58-1.53,  $p = 0.9$ ) and for *BRCA2* carriers was 0.82 (95%CI, 0.30-1.81,  $p = 0.7$ ). The SIR for colon cancer was 3.81 (95% CI 1.77 to 7.23) for women below the age of 50 (both genes combined) and was 0.65 (95% CI 0.37-1.06) for women aged 50 and above.

**Conclusions:** The risk of colorectal cancer is increased in female carriers of *BRCA1* and *BRCA2* mutations below the age of 50, but not in older women.



## Background

The *BRCA1* and *BRCA2* genes confer increased susceptibility to breast and ovarian cancer (1-5) and to a spectrum of other cancers (6-10). However, the risk for colorectal cancers associated with *BRCA1* and *BRCA2* mutations remains unclear. Previous studies which were designed to estimate the risk of colon cancer among mutation carriers were based on cross-sectional reviews of family histories of women with mutations and were susceptible to both selection bias and misclassification. The diagnoses of colorectal cancer were based on information provided by a family member and this approach may not always be accurate. Further, in previous studies, the mutation status of the colorectal cancer cases was unknown. We conducted a prospective study of the incidence of colorectal cancer in a cohort of *BRCA1* and the *BRCA2* mutation carriers. We followed 7,105 women with a *BRCA1* or *BRCA2* mutation for a mean of 5.5 years. The knowledge of colorectal cancer incidence rates has important implications for genetic counselors and their patients and for developing appropriate screening policies.

## METHODS

### Study Population

Eligible study participants were women at one of 50 centers in six countries from Canada, the United States or Europe who carry a deleterious *BRCA1* or *BRCA2* mutation. In most cases, genetic testing was offered initially to women who were affected either by breast or ovarian cancer. When a mutation in either *BRCA1* or *BRCA2* was found in a proband or in her relative, testing was offered to other at-risk women in her family, both affected and unaffected. In some cases, mutation testing was offered directly to unaffected women when no affected family

member was available for testing. The criteria for genetic testing varied from center to center, but all participating facilities offered testing to both affected and unaffected women. Mutation detection was performed using a range of techniques, but all abnormal nucleotide sequences were confirmed by the direct Sanger sequencing. A woman was eligible for the study when the molecular analysis established that she was a mutation carrier. She was then asked to participate in this prospective study and to complete a baseline questionnaire. Participants were enrolled in the study from 1992 to 2010. The majority of participants completed the baseline questionnaire at the time of genetic testing or within one year of receiving their result. This is a dynamic cohort with ongoing accrual, and therefore, the lengths of follow-up varied from individual to individual. Participants completed a baseline questionnaire and at least one follow-up questionnaire, a minimum of two years following the baseline questionnaire. The baseline and follow-up questionnaires requested information regarding reproductive history, surgical history (including preventive oophorectomy and mastectomy) and all new diagnoses of cancers. All participants provided written informed consent for genetic testing and for participating in the prospective study. The ethics committees of all participating centers have approved the study. The 7,021 carriers were from 3878 different families. Of 7,021 women, 6 women had data missing on key variables (e.g. date of birth, date of questionnaires, gene mutation) and were excluded, leaving 7,015 women in final analysis. An attempt was made to retrieve the pathology report for each of the incident cases of colorectal cancer.

### **Statistical analysis**

Women who had a history of breast cancer were eligible for follow-up but women with a history of ovarian cancer were considered ineligible. Women were followed from the date at the

baseline until the date of last questionnaire, the diagnosis of colorectal cancer, diagnosis of ovarian cancer or death from another cause. The risk of colorectal cancer in the *BRCA1* and *BRCA2* mutation carriers, relative to the general population was estimated with standardized incidence ratios (SIRs). The SIR was obtained by calculating the ratio of observed to expected number of new colorectal cancer cases, for all women combined and then separately for *BRCA1* and the *BRCA2* carriers. To do so, the observed and expected numbers of colorectal cancers were derived separately for carriers from each of six countries (Canada, United States, Poland, France, Italy, Norway). First, the annual age-standardized incidence rates of colorectal cancer were calculated with the count (numerator) and person-year at risk (denominator). The person-years at risk were calculated using the total number of *BRCA1* and *BRCA2* carriers multiplied with the average follow-up time in years. The person-years were subcategorized according to different countries and different age groups. The age-standardized incidence rates for each participating country were obtained from GLOBOCAN 2008 (Ferlay et al. 2010). The expected number of colorectal cancers was calculated by multiplying each age-standardized incidence rate of the country-specific general population by the observed person-years in our *BRCA* cohort and divided by 100,000 to get the expected numbers of colorectal cancers (indirect standardization method). The SIRs were also estimated for age subgroups <50 years and  $\geq 50$  years.

## RESULTS

7,015 women in our database of *BRCA1* and *BRCA2* carriers were followed for a mean of 5.5 years. The mean age of the cohort at study entry was 47.3 years (range, 30-74 years). 5,481 participants (78.13%) carried a *BRCA1* mutation, 1,474 (21.01%) carried a *BRCA2* mutation, and

60 participants (0.8%) carried both a *BRCA1* and *BRCA2* mutation. 2829 women (40%) had a past history of breast cancer.

We identified 22 new colorectal cancer cases among the *BRCA1* and *BRCA2* carriers versus 23.6 colorectal cancers expected (SIR = 0.93,  $p = 0.7$ ). Of the 22 colorectal cancer cases, 17 cases occurred in *BRCA1* carriers, versus 17.37 colorectal cancers expected (SIR = 0.97,  $p = 0.9$ ) and 5 cases occurred in *BRCA2* carriers versus 6.1 colorectal cancers expected (SIR = 0.82,  $p = 0.7$ )(table 1). The characteristics of the 15 colorectal cases for whom a pathology report was retrieved are presented in Table 2.

The mean age of diagnosis for the cases of colon cancer was 56.3 years (53.5 years for the *BRCA1* mutation carriers and 57.3 years for the *BRCA2* mutation carriers). For women above the age of 50, the annual incidence rate was 52 per 100,000 per year for *BRCA1* carriers and was 87 per 100,000 per year for *BRCA2* carriers (Table 3). For women below the age of 50, the annual incidence rate was 62 per 100,000 per year for *BRCA1* carriers and was 40 per 100,000 per year for *BRCA2* carriers. The risk of colon cancer was four times greater than expected for young women (aged 30-49) (SIR = 3.81; 95% CI 1.77-7.23). The risk of early onset colon cancer was significantly elevated only for *BRCA1* carriers. There were eight colorectal cancers in *BRCA1* carriers before age 50, the youngest occurred at age 32 and the others at ages 38, 41, 43, 46, 47, 48 and 49 years. There was no evidence of an increase in the risk of colorectal cancer for women aged above 50 years (14 cancers observed, 21.5 cancers expected).

In addition to the 22 new cases of colorectal cancer, there were single cases of cancer of the gallbladder and bile duct. Because of the small numbers of these cases, SIRs were not estimated. No case of stomach cancers was reported in the follow-up period.

## Discussion

We have estimated the risks for cancer of the colon, rectum, gallbladder, bile duct and stomach among women with a *BRCA1* or *BRCA2* mutation. There were only single cases of cancers of the gallbladder and bile duct (and no case of stomach cancer) and therefore it was not possible to do a formal analysis for these. In contrast, there were 22 cases of colorectal cancer, but the incidence was not in excess of what we would have expected by chance, based on population rates for the five countries. However, the risk of early-onset colon cancer was significantly increased for young *BRCA1* carriers.

Our findings are consistent with the studies of cancers the Breast Cancer Linkage Consortium [1-3] wherein the authors reported modest but significant increases in colorectal cancer risk in family members of families with a *BRCA1* or *BRCA2* mutation. The relative risk associated with a *BRCA1* mutation was 2.03 (RR = 2.03, 95% CI 1.45 to 2.85,  $P < .001$ ) (2). Easton et al, performed a comprehensive assessment of the cancer risks to *BRCA2* mutation carriers in these families. Significantly increased risks in carriers were observed for cancers of the stomach (RR = 2.59; 95% CI 1.46–4.61;  $P = .012$ ) and gallbladder and bile ducts (RR = 4.97; 95% CI 1.50–16.52;  $P = .03$ ) but not for colon cancer (RR = 1.43; 95% CI 0.79–2.58) and rectal cancer (RR = 1.11; 95% CI 0.48–2.60).

Lim et al, 1999 investigated 42 patients with colorectal cancer from 32 hereditary breast cancer kindreds and a general population colorectal cancer cohort was composed of 755 patients from a tumor registry. They found the mean age of onset for patients with colorectal cancer was 60 years (+/- 2 years) for hereditary breast cancer kindreds compared with 67 years (+/- 0.4 years)

for the general population ( $P = 0.0004$ ). A greater number of Stage I and fewer Stage IV cancers were seen in subjects with hereditary breast cancer compared with the general population ( $P = 0.01$ ). Overall BRCA-associated colorectal cancer had an earlier age of onset, lower tumor stage, and better survival rate than the general population.

In a recent study, 2,398 unselected patients with colorectal cancer and 4,570 controls from Poland were screened for three BRCA1 founder mutations. A BRCA1 mutation was present in 0.42% of unselected cases of colorectal cancer and in 0.48% of controls ( $OR = 0.8$ ;  $P = 0.8$ ). In keeping with our findings, BRCA1 mutations were more common than this (0.82%) in 851 cases who were diagnosed with colorectal cancer at age 60 or earlier ( $OR = 1.7$ ) but this was not significant ( $P = 0.3$ ). In a similar study conducted in Ashkenazi Jews, Niell et al 2004 identified a founder mutation in BRCA1 or BRCA2 in 24 of 1422 colon cancer cases and 20 of 1566 controls ( $OR = 1.24$ , 95% confidence interval [CI] = 0.68 to 2.26).

In a population-based series of 649 unselected incident cases of ovarian cancer, Risch et al identified 60 mutations among 515 women with invasive ovarian cancers, 39 in BRCA1 and 21 in BRCA2. The risk of colorectal cancer was increased 3-fold for relatives of cases carrying a BRCA2 mutation. The risk of stomach cancer was increased 6-fold among first-degree relatives of cases carrying a BRCA1 mutation, compared with relatives of non-carriers. We reported no incident case of stomach cancer in our cohort. Jakubowska et al [1] observed a strong association between co-occurrence of ovarian cancer and stomach cancer in a family and the presence of a BRCA2 mutation in Poland. The increased risk of stomach cancer in BRCA2 carriers was observed predominantly in males. Bermejo et al [5] conducted a large retrospective

population study in Swedish families eligible for BRCA1/2 testing. A 2-fold increased incidence of stomach cancer was observed; most individuals diagnosed with stomach cancer were men. The authors suggest an association of BRCA1 mutation carrier status in men with stomach cancer.

Our study provides the strongest evidence to date for an increased risk of colon cancer in young women with a BRCA1 or BRCA2 mutation. The primary strength of our study is that this is a large-scale prospective study in women confirmed to carry a BRCA1 or BRCA2 mutation. Many previous studies used a historical cohort design whereby genetic testing took place after the diagnoses of the incident cancers. Historical cohort studies are subject to bias because women who experience the endpoint of interest (colon, rectal and stomach cancer) may be more (or less) likely to undergo testing than healthy women because of local genetic testing criteria or high mortality. Our study has a number of limitations pertaining to both the estimation of the incidence and overall survival. Our cohort study includes only women and therefore, we cannot estimate the incidence of colorectal cancer in males. The number of incidence cases was relatively small ( $n = 22$ ) and this limits the precision of the risk estimates, especially for subgroups. We were able to retrieve a pathology report or medical record on 18 of the cases (82%) and the remainder were based on personal reporting.

The U.S. Preventive Services Task Force (USPSTF) recommends general population screening for colorectal cancer using high-sensitivity fecal occult blood testing, sigmoidoscopy, or colonoscopy beginning at age 50 years and continuing until age 75 years (11). However, they recommend that people at higher risk of developing colorectal cancer should begin screening at a younger age, and may need to be tested more frequently. Based on the results reported here, we



recommend screening for colon cancer for women with a BRCA1 mutation be initiated at age 40.

There are insufficient data to make a recommendation for BRCA2 carriers that is different from that of the general population.

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Table 1: Observed and expected number of colorectal cancer in *BRCA1* and *BRCA2* carriers according to the contributing country.

Country	<i>BRCA</i> status	N	Person-years	Rate Per 100,000	Observed cancers	Expected cancers	SIR (95% CI)	p-value (two-tailed)
Canada	All	1760	10494	72.23	8	9.16	0.87 (0.40-1.65)	0.7
	<i>BRCA1</i>	1012	6172	97.21	6	5.13	1.17 (0.47-2.43)	0.6
	<i>BRCA2</i>	725	4222	47.37	2	3.96	0.50 (0.08-1.67)	0.3
USA	All	1853	10071	69.50	7	6.64	0.95 (0.49-1.6)	1
	<i>BRCA1</i>	1253	7027	71.15	5	4.47	1.12 (0.41-2.4)	0.7
	<i>BRCA2</i>	577	2934	68.16	2	2.1	0.95 (0.16-3.1)	1
Austria	All	141	704	142.04	1	0.26	3.85 (0.19-19)	0.2
	<i>BRCA1</i>	107	548	-	0	0.18	-	-
	<i>BRCA2</i>	20	118	847.45	1	0.07	14.29 (0.71-70.4)	0.07
Poland	All	2475	11718	34.13	4	4.28	0.93 (0.29-2.2)	0.9
	<i>BRCA1</i>	2468	11596	34.49	4	4.23	0.94 (0.30-2.3)	0.9
	<i>BRCA2</i>	7	22	-	0	0.01	-	-
Norway	All	682	5160	38.76	2	4.53	0.44 (0.07-1.60)	0.2
	<i>BRCA1</i>	556	4392	45.54	2	3.76	0.53 (0.09-1.75)	0.3
	<i>BRCA2</i>	126	803	-	0	0.81	-	-
All countries	All	7015	38646	56.93	22	23.6	0.93 (0.59-1.38)	0.7
	<i>BRCA1</i>	5481	30191	56.31	17	17.37	0.97 (0.58-1.53)	0.9
	<i>BRCA2</i>	1474	8208	60.96	5	6.1	0.82 (0.30-1.81)	0.7

Abbreviations: SIR, standardized-incidence ratio; CI, confidence interval

Table 2: Characteristics of the incidence cases of colorectal cancer in BRCA mutation carriers

Pedigree number	Age	BRCA status	Tumor site	Tumor pathology	Tumor Grade	Duke's stage
CR1973	46	BRCA1	Anal canal	Squamous cell carcinoma	Grade 2	Unk
UU2802	51	BRCA1	Unknown	Adenocarcinoma	Unk	Unk
BCW141	57	BRCA1	Right colon	Adenocarcinoma	Grade 2	Duke B
BC169	48	BRCA1	Right colon	Adenocarcinoma	Grade 3	Duke C
CI1407	73	BRCA1	Metastatic, PSU	Adenocarcinoma	Unk	Unk
NR1575	32	BRCA1	Left colon	Adenocarcinoma	Grade 2	Duke C
NR1353	55	BRCA1	Transverse colon	Adenocarcinoma	Grade 3	Duke C
PD121177	61	BRCA1	Unknown	Adenocarcinoma	Grade 2	Unk
PD56009	44	BRCA1	Anal canal	Squamous cell carcinoma	Unk	Unk
ED37623	65	BRCA1	Rectum	Adenocarcinoma	Grade 1	Duke C
HM1030	56	BRCA1	Anal canal	Squamous cell carcinoma	Grade 3	Unk
AUF089	62	BRCA2	Sigmoid colon	Adenocarcinoma	Grade 2	Duke B
SB42233	65	BRCA2	Transverse colon	Adenocarcinoma	Grade 2	Duke B
CLC018	77	BRCA2	Sigmoid colon	Adenocarcinoma	Grade 2	Duke A
VT9729	55	BRCA2	Anal canal	Squamous cell carcinoma	Grade 1	Duke A

Abbreviations: PSU, primary site unknown; Unk, unknown

Table 3: Observed and expected number of colorectal cancer in *BRCA1* and *BRCA2* carriers according to age groups.

<i>BRCA</i> status	Age (years)	N	Person -years	Rate per 100,000	Observed Cancers	Expected cancers	SIR (95% CI)	p-value (two-tailed)
All	30-49	3194	15466	51.73	8	2.10	3.81 (1.77-7.23)	0.001
	50-74	3821	23180	60.40	14	21.50	0.65 (0.37-1.06)	0.09
<i>BRCA1</i>	30-49	2638	12848	62.27	8	1.68	4.76 (2.21-9.04)	<0.001
	50-74	2843	17342	51.90	9	15.68	0.57 (0.28-1.05)	0.07
<i>BRCA2</i>	30-49	554	2486	-	0	0.39	-	-
	50-74	980	5722	87.38	5	5.70	0.87 (0.32-1.94)	0.8

Abbreviations: SIR, standardized-incidence ratio; CI, confidence interval



# Cellular Transport Gene Variants Are Associated With Susceptibility to Epithelial Ovarian Cancer Risk and Histopathologic Subtypes

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## Abstract

**Background.** Epithelial Ovarian Cancer (EOC) is a lethal gynecologic malignancy and the fifth cause of cancer mortality in the U.S. females. Recently, cellular transport processes were implicated in the pathogenesis of EOC. We hypothesized that variation in cellular transport genes is associated with the EOC risk.

**Methods.** In a discovery set of 7931 EOC cases and 9206 controls, we investigated 4200 SNPs in ??? genes, 472 of which showed associations up to the significance level of  $p < 0.05$ . In a replication set we genotyped 305 of the most significant SNPs in 10,243 EOC cases and 16,932 controls so the combined dataset consisted of 18,736 EOC cases and 23,448 controls (of European ancestry). The 305 SNPs, from 120 genes were genotyped using an Illumina Infinium iSelect BeadChip as part of the Collaborative Oncological Gene-environment Study (COGS). Both invasive cancers combined and the four main histological subtypes (serous [ $n=8,372$ ], endometrioid [ $n=2,068$ ], clear cell [ $n=1,025$ ] and mucinous [ $n=943$ ]) were analyzed. SNP analyses were conducted using unconditional logistic regression under a log-additive model. All analyses were adjusted for study site and population substructure.

**Results.** The strongest evidence of an association for invasive cancers combined (INV) was observed for SNP rs17216603 in the iron transporter *HEPH* ( $OR=0.85$ ,  $95\%CI=0.77-0.93$ ,  $P=2.55 \times 10^{-4}$ ), which was also the most significant SNP associated with the serous subtype ( $P=1.99 \times 10^{-4}$ ), and associated with the borderline/low malignant potential (LMP) tumors ( $P=0.0206$ ). The most significant association for endometrioid EOC was rs11563251 near the *UGT1A* gene cluster ( $OR=0.82$ ,  $95\%CI=0.73-0.92$ ,  $P=6.59 \times 10^{-4}$ ). The most significant associations for mucinous subtype was rs681309 near the *SLC25A45* ( $OR=0.89$ ,  $95\%CI=0.81-0.97$ ,  $P=0.012$ ). This SNP was also associated with the endometrioid subtype ( $P=0.0035$ ) and INV EOC ( $P=0.0294$ ). The most significant association with clear cell carcinoma was rs7562766 located near *CAPN13* ( $OR=0.86$ ,  $95\%CI=0.79-0.95$ ,  $P=0.0014$ ). One more SNP in *CAPN13* was significant in the clear cell subtype only. Association with the rs9908917 in the intron of *SLC39A11* was observed in LMP ( $OR=0.85$ ,  $95\%CI=0.77-0.93$ ,  $P=3.9 \times 10^{-4}$ ). This SNP was also associated with the serous subtype ( $P=0.0123$ ) and INV EOC ( $P=0.0144$ ). Five additional SNPs in the *SLC39A11* were significant across different histologic subtypes. Interestingly, rs1804495 in *SERPINA7* was associated with serous, mucinous, clear cell, INV and LMP subgroups ( $P < 0.05$ ).

**Conclusion.** Our results, generated on a large cohort of women, suggest specific associations between inherited cellular transport gene variants and risk of different EOC histologic subtypes.

## Introduction

Epithelial ovarian carcinoma (EOC) is the most common gynecological malignancy and the leading cause of gynecological cancer-related mortality in the United States (US) and other developed nations [1]. Early stage EOC is accompanied by vague, non-specific symptoms and is difficult to detect. Importantly, there are no EOC screening or early detection strategies that have been proven useful [2]. As a consequence, approximately 60% of cases are diagnosed at later stages (III-IV) with a 5 year overall survival of less than 20% [3]. Typical management of advanced ovarian cancer involves surgical cytoreduction followed by platinum-based chemotherapy [4]. Initially the majority of patients respond to the chemotherapy but at least half of all patients recur within 4 years [5]. Given these grim statistics, improved understanding of the disease etiology is critical to reduce the morbidity and mortality associated with ovarian cancer.

A major deterrent in the detection and treatment of ovarian cancer is our poor understanding of the genetic and biological basis for this disease. Disruptions in the cellular transport processes, such as metal ion transport through the cellular membranes, has been associated with increased risk of a number of cancers including breast [6-8], prostate [9], liver [10], colorectal [11], thyroid [12] and neuroblastoma [13]. Iron is essential for erythropoiesis [14] and the respiratory chain functioning where it plays a key role in electron transport, in the form of iron-sulphur clusters or in haem centers [15]. However, excess iron and copper may promote the formation of reactive oxygen radicals possibly leading to organ damage and supporting cancer growth [16]. Partially reduced oxygen species (PROS) formed as a byproduct in the mitochondrial respiratory chain, are rendered innocuous by the enzyme superoxide dismutase and converted to oxygen and hydrogen peroxide. Hydrogen peroxide is in itself stable but can react with iron or copper and form hydroxyl radicals which are extremely reactive and damaging to living tissues [17]. In concordance with these data, transport of the three most abundant transition metals in the human organism – iron, zinc and copper – was linked to the etiology of liver [10, 18], prostate [19-20] and lymphoma [21] cancers, although the biological basis for this association is poorly understood.

Despite the growing evidence of association between the cellular transport processes and cancer risk, the association of germline genetic variation in the cellular transport genes and EOC risk has not been studied. We hypothesized that single nucleotide polymorphisms (SNPs) in the cellular transport genes are associated with EOC risk and histopathology. In a discovery set of 7931 EOC cases and 9206 controls, we investigated 4200 SNPs in 100 genes, 305 of which showed associations up to the significance level of  $p < 0.05$ . This study describes the replication of these findings and we present the results of statistically significant associations with EOC risk with 305 SNPs in 120 genes in the large combined dataset of 18,736 EOC cases and 23,448 controls.

## **Materials and Methods**

### **Discovery Set Study Populations**

The Discovery set consisted of 7931 EOC cases and 9205 controls in total. We combined the results from two ovarian cancer GWAS from North America and the UK. Details of these studies have been previously published [22]. Briefly, the North American study comprised four case control studies genotyped using the Illumina 610-quad Beadchip Array™ (1,815 cases and 1,900 controls) and a single case-control study genotyped on the Illumina 317K and 370K arrays (137 cases and 142 controls). The UK study comprised four case-only studies genotyped on the Illumina 610-quad and two common control sets genotyped on the Illumina 550K array (1,817 cases and 2,354 controls). A second phase of the UK GWAS comprised an additional ten case-control studies (4,162 cases and 4,810 controls). The North American and UK studies were analysed separately and the results combined using standard fixed effects meta-analysis. SNPs were ranked according to the p-values for each of four analyses performed: North America study only (all histologies and serous histology), and combined GWAS meta-analysis (all histologies and serous histology). The minimal ranking for each SNP was obtained across the four sets of results. We acquired the design score for each SNP using the Illumina Assay Design Tool and removed SNPs that were redundant or predicted to perform poorly. In total, 322 SNPs were included on the COGS custom genotyping array and 307 passed QC.

### **Replication/Combined Set Study populations**

The replication set included an additional 10,243 cases and 16,932 controls. The combined set consisted of a total of 47,517 samples from 44 sites in OCAC were genotyped of which 44,312 passed QC, including 18,174 (10,316 serous) cases and 26,138 controls [22]. HapMap/1000 Genomes Project samples for European (CEU, n=60), African (YRI, n=53) and Asian (JPT+CHB, n=88) were also genotyped.

### **SNP Selection**

A total of 120 cellular transport-related genes were identified through review of the published literature ([www.pubmed.gov](http://www.pubmed.gov)) and pathways described in the Cancer Genome Anatomy Project. There were over 4200 SNPs in these 120 genes in the Illumina 550K and 610quad chip, of which 427 were statistically significantly associated with EOC risk ( $p < 0.05$ ) in the discovery set. The final selection of immunomodulatory -related SNPs for genotyping in COGS was informed by ranking of minimal p-values from the US GWAS and/or US/UK GWAS meta-analysis data. The top 305 SNPs in 120 genes were selected for genotyping in the replication set.

### **SNP genotyping**

Genotyping was conducted using an Illumina Infinium iSelect BeadChip in two centers for OCAC - Genome Quebec (n=25,782) and the Mayo Clinic Medical Genome Facility (n=18,707). Each 96-well plate contained 750 ng genomic DNA (or 1,500 ng whole-genome amplified DNA). Raw intensity data files for all consortia were sent to the COGS data co-ordination centre at the University of Cambridge for centralized genotype calling and QC. Genotypes were called using GenCall {Kermani, 2008 #10972}. Initial calling used a cluster

file generated using 270 samples from Hapmap2. These calls were used for ongoing QC checks during the genotyping. To generate the final calls used for the data analysis, we first selected a subset of 3,018 individuals, including samples from each of the genotyping centres, each of the participating consortia, and each major ethnicity. Only plates with a consistent high call rate in the initial calling were used. HapMap and 1000 Genomes Project samples, along with ~160 samples that were known positive controls for rare variants on the array were used to generate a cluster file that was then applied to call the genotypes for the remaining samples using GenCall.

### **Sample QC**

One thousand two hundred and seventy three OCAC samples were genotyped in duplicate. Genotypes were discordant for greater than 40 percent of SNPs for 22 pairs. For the remaining 1,251 pairs, concordance was greater than 99.6 percent. In addition we identified 245 pairs of samples that were unexpected genotypic duplicates. Of these, 137 were phenotypic duplicates and judged to be from the same individual. We used identity-by-state to identify 618 pairs of first-degree relatives. Samples were excluded according to the following criteria: 1) 1,171 samples with a conversion rate of less than 95 percent; 2) 169 samples with heterozygosity >5 standard deviations from the intercontinental ancestry specific mean heterozygosity; 3) 67 samples with ambiguous sex; 4) 294 samples with the lowest call rate from a first-degree relative pair 5) 1,604 samples that were either duplicate samples that were non-concordant for genotype or genotypic duplicates that were not concordant for phenotype. Thus, a total of 44,312 subjects including 18,174 cases and 26,138 controls were available for analysis.

### **SNP QC**

Robust genotyping quality control guidelines were used to ensure accurate genotyping. SNPs were excluded according to the following criteria: (1) SNPs without a genotype call; (2) monomorphic SNPs; (3) SNPs with a call rate less than 95 percent and MAF > 0.05 or call rate less than 99 percent with MAF < 0.05; (4) SNPs showing evidence of deviation of genotype frequencies from Hardy-Weinberg equilibrium ( $p < 10^{-7}$ ); (5) SNPs with greater than 2 percent discordance in duplicate pairs. Overall, 305 of the 320 (95.3%) selected transport SNPs passed QC.

### **Statistical methods**

Analysis of the combined COGS data set

We used the program LAMP {Sankararaman, 2008 #6445} to assign intercontinental ancestry based on the genotype frequencies for these three populations. Subjects with greater than 90 percent European ancestry were defined as European (n=39,944) and those with greater than 80 percent Asian and African ancestry were defined as being Asian (n=2,388) and African respectively (n=387). All other subjects were defined as being of mixed ancestry (n=1,770). A set of 37,000 unlinked markers was used to perform principal components

analysis within each major population subgroup (using a programme available at <http://ccge.medschl.cam.ac.uk/software/>).

Unconditional logistic regression treating the number of alternate alleles carried as an ordinal variable (log-additive, co-dominant model) was used to evaluate the association between each SNP and ovarian cancer risk. A likelihood ratio test was used to test for association, and per-allele log odds ratios and 95 percent confidence limits were estimated.{Xing, 2012 #10925}. Separate analyses were carried out for each ancestry group. The model for European subjects was adjusted for study and population substructure by including study-specific indicators and the first five eigenvalues from the principal components analysis in the model. For analysis of the Asian and other ancestry groups, the first five ancestry-specific principal components were included in the model, and one principal component was included in the model for analysis of subjects of African ancestry. These cut-offs were based on the position of the inflexion of the principal components scree plot.

In order to assess the magnitude of confounding due to cryptic population substructure, we tested for association for the 147,722 SNPs included on the genotyping array that not been selected as candidates for ovarian cancer susceptibility. In analyses restricted to European subjects and adjusted only for study, there was a small inflation of the test statistic for ( $\lambda = 1.13$  equivalent to  $\lambda = 1.007$  for a study of 1000 cases and 1000 controls). This was reduced to 1.078 ( $\lambda_{1000} = 1.004$ ) after adjusting for five principal components. Heterogeneity of odds ratios between studies was tested with Cochran's Q statistic.

To maximize statistical power, the "combined" COGS dataset was used to perform SNP-specific analyses for all invasive EOC, the four main histological subtypes (serous, endometrioid, clear cell and mucinous), tumors of low malignant potential (LMP) and for all the ethnicities. OR specific for each histological subtype was estimated by comparing cases of each subtype to all available controls as the reference. We also stratified the analysis for the top SNPs in all invasive EOC and serous histological subtype by risk factors for ovarian cancer including age at diagnosis/interview, age at menarche, parity, oral contraceptive use and family history. Linear trends for age at diagnosis/interview and menarche were determined.

**Results**

**Table 1: Clinical characteristics of cases and controls.**

**Table 2: Statistically significant associations with EOC risk by tumor behavior and histologic subtype in European subjects**

**Table 3: Statistically significant associations with serous EOC risk in European, Asian and African American subjects**

**Table 4: GxE interactions in all invasive EOC combined in European women**

Supplementary Table 1: The Discovery set?

Supplementary Table 2: Statistically significant associations for the 85 SNPs which remained significant in the discovery, replication and combined datasets.

We genotyped 305 SNPs from 120 cellular transport genes (Supplementary Table 2), in 18,736 EOC cases and 23,448 controls of European ancestry. Both invasive cancers combined and the four main histological subtypes (serous [n=8,372], endometrioid [n=2,068], clear cell [n=1,025] and mucinous [n=943] were analyzed. The mean age at diagnosis/interview for women of European ancestry was 55 (std=11) years (Table 1). Distribution of risk factors (Table 1). As expected, the proportion of serous histological subtype (58%) was higher than the other subtypes (15% for endometrioid, 7% for mucinous and 7% for clear cell) (Table 1).

Eighty-four SNPs remained statistically significantly associated with EOC risk on replication and in the combined dataset (Supplementary Table 2).

The strongest evidence of an association for invasive cancers combined (INV) was observed for SNP rs17216603 in the iron transporter *HEPH* (OR=0.85, 95%CI=0.77-0.93,  $P=2.55 \times 10^{-4}$ ), which was also the most significant SNP associated with the serous subtype ( $P=1.99 \times 10^{-4}$ ), and associated with the borderline/low malignant potential (LMP) tumors ( $P=0.0206$ ). The most significant association for endometrioid EOC was rs11563251 near the *UGT1A* gene cluster (OR=0.82, 95%CI=0.73-0.92,  $P=6.59 \times 10^{-4}$ ). The most significant associations for mucinous subtype was rs681309 near the *SLC25A45* (OR=0.89, 95%CI=0.81-0.97,  $P=0.012$ ). This SNP was also associated with the endometrioid subtype ( $P=0.0035$ ) and INV EOC ( $P=0.0294$ ). The most significant association with clear cell carcinoma was rs7562766 located near *CAPN13* (OR=0.86, 95%CI=0.79-0.95,  $P=0.0014$ ). One more SNP in *CAPN13* was significant in the clear cell subtype only. Association with the rs9908917 in the intron of *SLC39A11* was observed in LMP (OR=0.85, 95%CI=0.77-0.93,  $P=3.9 \times 10^{-4}$ ). This SNP was also associated with the serous subtype ( $P=0.0123$ ) and INV EOC ( $P=0.0144$ ). Five additional SNPs in the *SLC39A11* were significant across different histologic subtypes. Interestingly, rs1804495 in *SERPINA7* was associated with serous, mucinous, clear cell, INV and LMP subgroups ( $P<0.05$ ).

We have conducted exploratory analyses for other ethnicities and serous EOC. 14 SNPs showed significant associations in Asian and African women, each (compared to 16 significant associations in women of European descent). Six of the SNPs in Asian women were also significant in European women compared to two of the 14 SNPs in African women. The two top SNPs in women of Asian ancestry (rs17216603 in *HEPH* and rs1552846 in *SLC39A11*) were shared with the NHW women. The *SLC25A45* rs681309 was also shared. *SERPINA7* rs1804495 was borderline significant ( $P=0.0503$ ) likely due to a small sample size. The most significant association in women of African ancestry was noted at the SNP rs6488840 in the Microsomal glutathione S-transferase 1 (*MGST1*) gene. In our study, *MGST1* rs6488840 was associated with statistically significantly reduced INV EOC risk in women of African ancestry (OR=0.55;  $P=0.0035$ ). This SNP was also borderline significant in women of European (OR=0.95;  $P=0.042$ ), but not Asian, ethnicity. However, the power to detect associations in Africans and Asians was low due to the small sample sizes of these populations.

Finally, we attempted to conduct the gene-environment (GxE) interaction analysis for the 28 SNPs that showed statistical significance with INV EOC in European women. This analysis included all invasive EOC combined in women of European descent in order to maximize the sample size. The following environmental variables were included in the analysis (Table 4), totaling 16 significant GxE interactions with 13 SNPs in 8 genes ( $p < 0.05$ ): oral contraceptives use (OC) – 1 interaction; being obese (OB) – 1 interaction; the number of full births (FB) – 1 interaction; age at menarche (MAGE) – 1 interaction; HRT use (HRT) – 5 interactions; endometriosis (endo) – 2 interactions; and family history of female breast and/or ovarian cancers (FH) – 5 interactions. No interactions were noted for being overweight (OW). Of the aforementioned 16 significant GxE interactions, five SNPs were in the *SLC35F3* and 2 SNPs were in the *MGST1*. Remaining genes with one SNP each included *ACSL1*, *SERPINA7*, *SLC30A1*, *SLC30A8*, *SLC39A11* and *TNFRSF21*.

## Discussion

In this work, we aimed to elucidate whether germline SNPs in the cellular transport genes are associated with the EOC risk and histopathology. We report that 85 SNPs remained statistically significant ( $p < 0.05$ ) in one or more histologic subtypes (Table 2). Multiple associations were noted with rs17216603 in *HEPH* (serous, INV and LMP subgroups) which was the most significant SNP in serous and INV in women of European and Asian ancestry; rs9908917 in *SLC39A11* (serous, INV and LMP subgroups) which was the most significant SNP in LMP; and rs681309 in the intergenic region near *SLC25A45* (mucinous, endometrioid and INV subgroups) which was the most significant SNP in mucinous. Interestingly, rs1804495 in *SERPINA7* showed associations with five out of six subgroups (serous, mucinous, clear cell, INV and LMP). The most significant association for the endometrioid subtype was with the *UGT1A* rs11563251, and with the clear cell subtype with the *CAPN13* rs7562766. The *UGT1A* and *CAPN13* SNPs were only associated with one histologic subtype each. Our results suggest that metal ion (primarily iron, zinc) and calcium transport, as well as mitochondria-related transport, thyroid hormones and glucuronidation may play a role in the EOC etiology across different histopathologic subtypes. Additional research in these venues is thus highly warranted as it may shed light on the currently poorly understood EOC etiology.

Hephaestin (*HEPH*) gene encodes a transmembrane copper-dependent ferroxidase (*HEPH* protein) responsible for dietary iron transport from the intestinal enterocytes into the blood stream [23-25]. While the ferrous ( $\text{Fe}^{2+}$ ) iron is transported through the basolateral enterocyte membrane into the circulation via the protein ferroportin, this form of iron is highly toxic. *HEPH* is coupled with the ferroportin and is responsible for the ferrous iron reoxidation to its ferric ( $\text{Fe}^{3+}$ ) state [26-27] that can be utilized by the body. *HEPH* expression is up-regulated by systemic and dietary iron deficiency [25]; conversely, sex-linked anemia (*s/a*) mice (possessing a naturally occurring germline mutation in the *HEPH* gene) develop an iron-deficiency anemia despite adequate dietary iron intake [24]. In these mice, iron accumulates in the enterocytes but is not transported into the circulation leading to systemic anemia. Taken together, these data suggest that *HEPH* protein is one of the direct modulators of iron intake from the enterocytes into the blood stream. As such, *HEPH* serves as a “gatekeeper” of iron available to the human organism. Although *HEPH* is mainly expressed



in the small intestine and the colon, its expression has also been detected in the lung, kidney, stomach, skin, nervous system, pancreas, placenta and other tissues [24, 28-29]; however, its role in these tissues is not clear. Similarly, the role of iron homeostasis in cancer progression is yet to be deciphered. It was reported that depletion of the iron storages in the cells causes cell cycle arrest and apoptosis, limits the rate of DNA synthesis, and down-regulates expression of various carcinogenic kinases such as cyclin-dependent kinases (CDKs) and the cyclins itself [30]. Additionally, iron is known to facilitate generation of reactive oxygen species (ROS) that have a direct connection to cancer [31]. Thus, it is biologically plausible that iron homeostasis may modulate cancer progression via disruptions in the cell cycle and/or generation of ROS. Specifically, depleted iron stores may lead to decreased rates of cancer growth. In concordance with this intriguing hypothesis, our bioinformatics simulated data indicated that rs17216603 may lead to a reduction or loss of the HEPH function, thus potentially altering (diminishing) systemic iron adsorption. Hence, it is tempting to suggest that women who carry at least one A-allele in the rs17216603 have decreased serous EOC risk due to diminished iron stores in the ovarian epithelium and/or the circulation, compared to women who are homozygous for the major G-allele. It is also possible that HEPH has other carcinogenic role(s) in the ovarian epithelium (unrelated to iron metabolism), such as kinase signaling, although at present there is no data to support or reject this hypothesis. Additionally, studies show that copper deficient mice have dramatically reduced levels of HEPH expression, diminished ferroxidase activity and shortened half life, indicating that copper is required for proper HEPH synthesis and/or function [32-33]. However, the relevance of this observation to our reported finding is unknown. Although intriguing and novel, verification of the aforementioned hypotheses will require extensive functional and epidemiological work.

Solute carrier family 39, member 11 (SLC39A11) belongs to a family of metal ion transporters. It is integrated in the cellular membrane and may act as a zinc-influx transporter (by similarity to other members with established zinc-transport activity such as A1 and A10), although the exact functions of the member A11 are yet to be experimentally established [34]. Members of the SLC39 family may also transport other metal ions, such as iron, copper, cadmium and manganese [35]. There is some experimental evidence that *SLC39A7* (Zip7) releases zinc from the endoplasmic reticulum and might be required for tyrosine kinase activation [36]. There are multiple reports of different SLC39 members being involved in cancer. For example, *SLC39A1* was reported [37] to act as a tumor suppressor gene in the prostate tumor cells by allowing zinc ions to accumulate in the prostate epithelium. The authors concluded that silencing the Zip1 (a protein product of the *SLC39A1*) leads to decreased intracellular concentration of zinc, loss of the tumor suppression and increased prostate cancer growth. *SLC39A4* (Zip4) was reported [38] to be significantly overexpressed in pancreatic cancer cells, which leads to the 2-fold increased proliferation and increased zinc uptake *in vitro*. Consequently, *in vivo* animal models with forcefully enhanced Zip4 expression demonstrated over 7-fold increased tumor weight, peritoneal dissemination and ascites incidence. These data suggest that Zip4 may facilitate pancreatic cancer proliferation. *SLC39A10* (Zip10) was involved in the invasive behavior of breast cancer cells suggesting that zinc and ZIP10 play an essential role in the migratory activity of highly metastatic breast cancer cells, and

suggest ZIP10 as a possible marker for the metastatic phenotype of breast cancer and a promising target of novel treatment strategies [39]. In the present study, rs9908917 in *SLC39A11* conferred modestly, albeit statistically significant decreased risk for serous, LMP and INV EOC. Importantly, five additional SNPs in the *SLC39A11* (rs1552846, rs1177982, rs8068946, rs9905659 and rs9913553) were significant across different histologic subtypes. Importantly, A11 is one of the most understudied *SLC39* members and we could not find any information on its specific function(s) in cancer. Given our results obtained using a very large cohort of women, such studies are highly warranted.

Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7 (*SERPINA7*), also known as thyroxine-binding globulin (TBG), is a protein that binds thyroid hormones thyroxine (T4) and 3,5,3'-triiodothyronine (T3) in circulation [40]. Numerous mutations in *SERPINA7* have been identified [41-43] leading to partially or completely absent TBG production. Although the hallmark of TBG deficiency is abnormally low T3 and T4 combined with normal thyroid stimulating hormone (TSH) values, the affected carriers are clinically asymptomatic [41]. This is because the amount of the unbound (active) thyroid hormone is not affected by the impaired TBG production enough to cause an overt thyroid dysfunction. The specific role of *SERPINA7* in cancer etiology has not been established; however, thyroid hormones may support cancer growth [44]. Thus, it is conceivable that impaired (diminished) TBG production in the minor allele rs1804495 carriers may lead to a slight elevation in the free T3 and/or T4 which, while clinically insignificant, may promote EOC growth if persists for many years. We note that in our study, rs1804495 confers statistically significantly increased risk for LMP, INV, serous and clear cell, but decreased risk for mucinous EOC. This observation is consistent with the previously published studies that emphasize on the differences between the mucinous disease and other EOCs [45-46]. In summary, our results suggest involvement of the thyroid hormone metabolism in EOC etiology across different histological subtypes and calls for additional studies on this topic.

Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 45 (*SLC25A45*) belongs to the family of nuclear-encoded membrane-embedded proteins that catalyze the transport of solutes across the inner mitochondrial membrane [47]. While substrates for the SLC25 family carriers include ADP/ATP, amino acids (glutamate, aspartate, lysine, histidine, and arginine), malate, ornithine, and citrulline [48], the predominant substrate(s) for the A45 specifically remain to be experimentally established [49]. Although specific functions of the SLC25A45 are unknown, it was reported that A45 is closely related to another SLC25 member, A29 [48]. SLC25A29 together with SLC25A20 are known to be responsible for transport of long-chain fatty acids such as palmitoylcarnitine and acylcarnitine [50]. It is thus possible that SLC25A4 is also involved in the transport of long-chain fatty acids. SLC25A45 is known to be expressed in the skeletal muscle, intestine, brain, and testis [49]. In our study, rs681309 in the *SLC25A4* conferred modest although statistically significantly decreased risk for mucinous, endometrioid and all INV EOC combined. Thus, our results warrant additional studies on the role of *SLC25A4* in particular, as well as mitochondria in general, in the EOC etiology.

UDP glucuronosyltransferase 1 family, polypeptide A cluster (*UGT1A*) represents a complex locus that encodes nine human UDP-glucuronosyltransferases. The locus includes thirteen unique alternate first exons followed by four common exons. Four of the alternate first exons are considered pseudogenes. Each of the remaining nine 5' exons may be spliced to the four common exons, resulting in nine proteins with different N-termini and identical C-termini. Each first exon encodes the substrate binding site, and is regulated by its own promoter [51]. UDP-glucuronosyltransferase (UGT) enzymes are localized to endoplasmic reticulum (ER) and catalyze glucuronidation, which represents a major phase II conjugation pathway in humans and is involved in the metabolism and excretion of endogenous and exogenous compounds. The process results in an increased polarity of the substrate that facilitates its excretion from the body through bile and urine [52]. This metabolic process is involved in the elimination of bilirubin, steroids, bile acids, toxic dietary components, and several drugs, including morphine, and irinotecan [53]. UDP-glucuronosyltransferase expression is primarily localized to the liver and bile ducts, but has also been detected in other organs such as stomach, colon, intestines, mammary glands, prostate and the lung [52]. Genetic variations in a sole member of the *UGT1A* family, *UGT1A1*, are involved in the inherited disorders of the bilirubin metabolism named such as Crigler-Najjar syndrome, which is manifested in the complete absence (type 1) or diminished (types 2-3) bilirubin glucuronidation and resulting impaired bilirubin excretion. While type 1 is a lethal disease in the absence of immediate liver transplantation, type 2 is treatable by prolonged induction therapy, while type 3 usually does not require medical intervention [52]. *UGT1A* locus was previously implicated in tumorigenesis. Strassburg et al [55] reported that the *UGT1A7\*3* allele exhibited a highly significant association with colorectal cancer (OR=2.39;  $P=0.02$ ), possibly due to diminished activity of the enzyme in the *UGT1A7\*3* carriers towards all tested substrates. Same group [56] later reported that pancreatic cancer was associated with the low detoxification activity *UGT1A7\*3* allele (OR=1.98;  $P = 0.003$ ). The association of *UGT1A7\*3* was especially strong in smokers with pancreatic carcinoma who were younger than 55 years (OR=4.7;  $P = 0.0009$ ). They also suggested [57] that differential down-regulation of the *UGT1A* locus may be an early event in hepatocarcinogenesis. Giuliani et al [58] determined that while abundant *UGT1A* expression was detected in the normal urinary bladder and intestinal epithelia, it was virtually absent in the corresponding tumor tissues. The authors hypothesized that UGTs constitutive expression in the normal mucosa could protect these organs from carcinogens released in the bladder or introduced directly with the diet in the colon. Araki et al [59] suggested that the presence of *UGT1A7* polymorphisms was associated with lung cancer. Homozygous *UGT1A7\*3* is a possible risk factor for lung cancer, at least in the Japanese population. The most relevant finding to our study was reported by Duguay et al [54], who reported that lower expression of *UGT1A1* decreases the risk of endometrial cancer by reducing the excretion of 2-hydroxyestradiol, the antiproliferative metabolite of estradiol, in the endometrium. Interestingly, in our study, rs11563251 near the *UGT1A* cluster was associated with a statistically significantly decreased endometrioid EOC risk. No other EOC subtypes showed significant associations with this SNP. Since *UGT1A* genes are known to be expressed in the endometrial cancer, as well as different EOC subtypes [60], and due to their resemblance to the endometrial

tissue, we suggest that further evaluation of the role of the UGT1A member in the endometrioid EOC is highly warranted.

Calpain 13 (CAPN13) is a protein in the family of well-conserved, calcium-dependent, non-lysosomal cysteine proteases (proteolytic enzymes) expressed ubiquitously in mammals and many other organisms [61]. Although the physiological role of calpains is still poorly understood, they have been shown to be active participants in processes such as cell mobility and cell cycle progression, as well as cell-type specific functions such as long-term potentiation in neurons and cell fusion in myoblasts [62]. Under these physiological conditions, a transient and localized influx of calcium into the cell activates a small local population of calpains (for example, those close to  $\text{Ca}^{2+}$  channels), which then advance the signal transduction pathway by catalyzing the controlled proteolysis of its target proteins. Other reported roles of calpains are in cell function, helping to regulate clotting and the diameter of blood vessels, and playing a role in memory. Calpains have been implicated in apoptotic cell death, and appear to be an essential component of necrosis [63]. Calpains are also involved in cancer. Mamoune et al [64] reported that pharmacological and molecular inhibitors of calpain-2 significantly reduce the motility and invasiveness of DU145 human prostate carcinoma cells both *in vitro* and *in vivo*, despite the fact that the overall tumor growth was not affected. In our study, rs7562766 near the *CAPN13* was associated with a statistically significantly decreased clear cell EOC risk. One more SNP in *CAPN13* (rs4952185) was significant in the clear cell subtype only. No other EOC subtypes showed significant associations with any of the CAPN13 SNPs.

### **Results in women of Asian ancestry**

Due to the limited sample size, we only analyzed the INV EOCs combined in women of Asian ancestry. The two top SNPs in this ethnic group (rs17216603 in *HEPH* and rs1552846 in *SLC39A11*) were shared with the NHW women. The *SLC25A45* rs681309 was also shared. *SERPINA7* rs1804495 was borderline significant ( $P=0.0503$ ) likely due to a small sample size. Overall, the INV EOC results observed in Asian women were highly concordant with the INV EOC results obtained in European women. We are tempted to hypothesize that the high level of concordance reflects similar genetic and biological disease etiology in Asian and European women; however, this provocative hypothesis remains to be tested.

### **Results in women of African ancestry**

Due to the limited sample size, we only analyzed the INV EOCs combined in women of African ancestry. The most significant SNP rs6488840 was in the Microsomal glutathione S-transferase 1 (MGST1) gene, which encodes a protein that catalyzes the conjugation of glutathione to electrophiles and the reduction of lipid hydroperoxides. This protein is localized to the endoplasmic reticulum and outer mitochondrial membrane where it is thought to protect these membranes from oxidative stress. The product of this gene is involved in the cellular defense against toxic, carcinogenic, and pharmacologically active electrophilic compounds [65]. *MGST1* overexpression has been demonstrated in various cancers (e.g., prostate cancer and lung cancer) and has been associated with high metastatic potential and chemoresistance. It has been

suggested to be an early marker of disease in colorectal and lung cancer [66]. In a study [65] *MGST1* was abundantly expressed in different EOC primary tumors, distant metastases and effusions. The authors hypothesized that oxidative response pathways are involved in the EOC etiology. In our study, *MGST1* rs6488840 was associated with statistically significantly reduced INV EOC risk in women of African ancestry ( $P=0.0035$ ); while this SNP was also associated with the INV and serous EOC in European women, the  $P$ -values were close to the limit of significance ( $P=0.048$  INV and  $P=0.042$  serous), possibly indicating weaker association. Other significant SNPs in women of African descent included various members of the *SLC39* family: *SLC39A11* (rs9905659 and rs16977431, both shared with European women) and *SLC39A8* (rs233807). Although the results were similar to the ones obtained for European and Asian women, overall the degree of concordance seemed weaker than the one observed for women of European and Asian descent. Whether this observation is an artifact of a small sample size in women of African descent, or a true finding reflecting biological and genetic differences in cancer etiology, remains to be established.

### Gene-Environment Interactions for INV EOC in European Women

We analyzed the Gene-Environment Interactions (GxE) for 28 significant INV SNPs in European women (Table 4). The following E were included: overweight (OW); obesity (OB); hormonal contraceptives use (OC); the number of full birth (FB); age at menarche (mage); hormonal replacement therapy use (HRT); personal history of endometriosis (end); and family history of breast and/or ovarian cancer (FH). Overall, statistically significant GxE were found for 13 SNPs in 8 genes. 5 of 13 significant SNPs were in the *SLC35F3* gene, and three of the 13 SNPs demonstrated significant association with two Es. No significant GxE were noted for being overweight (OW).

Solute carrier 35 member F3 (*SLC35F3*) gene encodes a putative transmembrane transporter which exact role has not been experimentally established [67]. *SLC35* is an extended diverse family of nucleotide-sugar transporters consisting of 6 distinct sub-families (A-F), each of which has up to five discovered members. Families E and F were discovered recently and are the most understudied subfamilies; thus, their exact substrates and functions in the transport are unknown [68]. The highest expression level of *SLC35F3* mRNA has been found in the adult cerebellum and brain, and - to lesser extent - in other tissues, including ovary [67]. In our study, *SLC35F3* SNPs were associated with OC use (rs10910292: OR=1.38;  $p=0.027$ ), HRT use (rs936411, rs10910354, rs10910358: ORs=1.12;  $p=0.039$ ), and FH (rs936411: OR=0.9;  $p=0.045$ ; rs10910360: OR=0.89;  $p=0.39$ ). Of those, rs936411 was associated with both the HRT and FH, although with disparate risk directions. These data suggest that *SLC35F3* might be involved in hormonal metabolism and transport. Although the role of *SLC35F3* in cancer has not been established, our data provides first evidence supporting the importance of future research on this topic.

Microsomal glutathione S-transferase 1 (*MGST1*) gene encodes a protein that catalyzes the conjugation of glutathione to electrophiles and the reduction of lipid hydroperoxides. Two SNPs in *MGST* were associated

with FH (rs4149186 and rs6488840: ORs=1.15; p=0.036), and one with HRT (rs6488840: OR=1.14; p=0.017) in our study. Of note, rs6488840 was associated with both the HRT and FH with identical risk profiles.

Solute liquid carrier family 30 member A8 (SLC30A8) is a transmembrane zinc transporter linked to insulin secretion [69]. Certain alleles of this gene may increase the risk for developing type 2 diabetes [70]. In our study, SLC30A8 rs10282940 was associated with the number of full birth (OR=1.06; p=0.014) and personal history of endometriosis (OR=0.77; p=0.045).

SLC30A1 and SLC39A11 both act as zinc influx transporters [71]. In our study, SLC30A1 rs12727395 (OR=1.14; p=0.0033) and SLC39A11 rs1552846 (OR=1.09; p=0.039) were associated with MAGE and OC use, respectively.

SERPINA7, a thyroid hormone transporter [40], was associated with FH (OR=0.82; p=0.023).

Long-chain-fatty-acid—CoA ligase 1 (ACSL1) encodes an isozyme of the long-chain fatty-acid-coenzyme A ligase family. Although differing in substrate specificity, subcellular localization, and tissue distribution, all isozymes of this family convert free long-chain fatty acids into fatty acyl-CoA esters, and thereby play a key role in lipid biosynthesis and fatty acid degradation [72]. In our study, ACSL1 rs10024579 was associated with end (OR=0.84; p=0.037).

Tumor necrosis factor receptor superfamily member 21 (TNFRSF21, also called DR6) is a member of the TNF-receptor superfamily. This receptor has been shown to activate NF- $\kappa$ B and MAPK8/JNK, and induce cell apoptosis. It is abundantly expressed in heart, brain, placenta, pancreas, thymus, lymph node and several non-lymphoid cancer cell lines [73]. Knockout studies in mice suggested that this gene plays a role in T helper cell activation, and may be involved in inflammation and immune regulation. In addition to its roles in regulating immune responses, DR6 may also be involved in tumor cell survival and immune evasion [74]. In our study, TNFRSF21 rs7751646 was associated with HRT (OR=0.89; p=0.027).

We note that GxE interactions are challenging to study and/or interpret due to their complexity and our insufficient understanding of the nature of those interactions. The authors are currently planning a study focused specifically on the GxE aspects of EOC.

The main strength of our study is a large sample size that allowed us to reach sufficient power to detect smaller associations typically observed on the SNP level. The weaknesses included the lack of the functional studies to establish and/or confirm the biological significance of the observed associations and/or the *HEPH* gene in general in the EOC etiology. The functional work is being planned by authors in the nearest future. Another weakness is a small sample size in each racial/ethnic category other than the Non Hispanic Whites. It is currently not clear whether genetic and/or biological differences between the racial groups contribute to the EOC etiology. However, because ovarian cancer health disparities are observed along the whole continuum of

the disease globally and in the U.S. [75], this topic is without a doubt important and deserves its own dedicated studies.

**In summary**, we report that genetic variation in the transmembrane transport genes contribute to the serous EOC risk across different histologic subtypes (Table X). This novel hypothesis-generating evidence suggests that disruptions in the metal ion (primarily iron, zinc) and calcium transport, as well as mitochondria-related transport, thyroid hormones and glucuronidation may play a role in the EOC etiology across different histopathologic subtypes. Functional studies are warranted to establish biological significance of these findings.

Table X: the top significant SNPs in the transport pathway. INV: all invasive EOC combined; LMP: low malignant potential / borderline tumors; Ser: serous; CC: clear cell; End: endometrioid; Muc: mucinous.

	INV	LMP	Ser	CC	End	Muc	Asian (INV)	African (INV)
<i>HEPH</i> rs17216603	<b>0.85 (0.77-0.93);</b> <b>2.55*10<sup>-4</sup></b>	<b>0.78 (0.63-0.97);</b> <b>0.0206</b>	<b>0.81 (0.73-0.91);</b> <b>1.99*10<sup>-4</sup></b>	0.77 (0.58-1.02); 0.07	0.9 (0.74-1.08); 0.2596	0.92 (0.7-1.21); 0.5565	<b>1.45 (1.15-1.83);</b> <b>0.0019</b>	0.76 (0.15-3.86); 0.74
<i>SLC39A11</i> rs9908917	<b>0.95 (0.92-0.99);</b> <b>0.014</b>	<b>0.85 (0.77-0.93);</b> <b>3.9*10<sup>-4</sup></b>	<b>0.94 (0.9-0.99);</b> <b>0.01012</b>	1.01 (0.91-1.13); 0.82	0.94 (0.87-1.02); 0.16	0.98 (0.87-1.1); 0.7	<b>1.25 (1.01-1.56);</b> <b>0.049</b>	1.23 (0.85-1.77); 0.27
<i>SLC39A11</i> rs1552846	<b>0.95 (0.91-0.98);</b> <b>0.0026</b>	1.00 (0.92-1.09); 0.92	<b>0.95 (0.91-0.99);</b> <b>0.0145</b>	0.95 (0.85-1.06); 0.34	<b>0.92 (0.86-1); 0.0465</b>	0.95 (0.85-1.06); 0.36	<b>1.34 (0.83-1.21);</b> <b>0.008</b>	0.81 (0.52-1.27); 0.36
<i>SLC39A11</i> rs1177982	0.97 (0.93-1); 0.069	1.01 (0.94-1.1); 0.7	<b>0.95 (0.91-0.99);</b> <b>0.0133</b>	1.05 (0.94-1.16); 0.38	0.95 (0.88-1.03); 0.23	1.01 (0.91-1.13); 0.84	0.83 (0.66-1.05); 0.12	0.61 (0.31-1.17); 0.13
<i>SLC39A11</i> rs8068946	1 (0.97-1.03); 0.91	1.03 (0.95-1.11); 0.46	1.02 (0.98-1.06); 0.37	<b>0.88 (0.8-0.97);</b> <b>0.0083</b>	0.98 (0.91-1.05); 0.48	0.98 (0.89-1.09); 0.73	0.95 (0.41-2.22); 0.9	0.92 (0.63-1.35); 0.68
<i>SLC39A11</i> rs9905659	1.01 (0.97-1.05); 0.56	1.05 (0.96-1.15); 0.29	1.03 (0.98-1.08); 0.27	<b>0.88 (0.79-1); 0.045</b>	0.98 (0.9-1.06); 0.61	1.01 (0.9-1.14); 0.83	1.06 (0.84-1.36); 0.61	<b>1.54 (1.0-2.35);</b> <b>0.047</b>
<i>SLC39A11</i> rs9913553	0.99 (0.94-1.04); 0.74	1.04 (0.93-1.17); 0.47	0.98 (0.92-1.04); 0.54	0.98 (0.84-1.13); 0.76	0.95 (0.85-1.06); 0.34	<b>1.16 (1-1.34);</b> <b>0.048</b>	0.97 (0.78-1.22); 0.82	1.24 (0.87-1.75); 0.23
<i>SERPINA7</i>	<b>1.05 (1-1.1);</b>	<b>1.14 (1.03-1.27);</b>	<b>1.06 (1-1.12);</b>	<b>1.21 (1.06-1.39);</b>	1.06 (0.96-	<b>0.85 (0.73-</b>	<b>0.81 (0.65-1.00);</b>	1.11 (0.75-

Rs1804495	<b>0.04187</b>	<b>0.016</b>	<b>0.045</b>	<b>0.0042</b>	1.17); 0.28	<b>1); 0.045</b>	0.0503	1.65); 0.602
SLC25A45 Rs681309	<b>0.97 (0.94-1); 0.029</b>	1.04 (0.97-1.12); 0.27	1 (0.96-1.04); 0.99	0.98 (0.89-1.07); 0.59	<b>0.91 (0.85-0.97); 0.0035</b>	<b>0.89 (0.81-0.97); 0.012</b>	<b>0.78 (0.63-0.98); 0.033</b>	0.87 (0.6-1.26); 0.41
UGT1A Rs11563251	0.95 (0.91-1); 0.0502	1.08 (0.96-1.2); 0.21	0.95 (0.89-1.01); 0.074	1.03 (0.89-1.18); 0.73	<b>0.82 (0.74-0.92); 6.8*10<sup>-4</sup></b>	1.06 (0.91-1.22); 0.49	0.73 (0.5-1.06); 0.102	1.22 (0.85-1.25); 0.28
CAPN13 rs7562766	0.99 (0.96-1.02); 0.49	0.97 (0.9-1.04); 0.45	1.01 (0.97-1.04); 0.79	<b>0.86 (0.79-0.95); 0.0014</b>	0.99 (0.93-1.05); 0.69	1.02 (0.93-1.12); 0.71	1.08 (0.82-1.43); 0.56	1.14 (0.73-1.79); 0.57
CAPN13 rs4952185	0.98 (0.95-1.01); 0.15	0.94 (0.87-1.01); 0.086	0.98 (0.94-1.02); 0.27	<b>0.88 (0.8-0.97); 0.0102</b>	1.02 (0.95-1.09); 0.67	0.97 (0.88-1.07); 0.53	0.85 (0.67-1.08); 0.19	0.78 (0.51-1.19); 0.25
MGST1 Rs6488840	<b>0.96 (0.93-1); 0.048</b>	1.0 (0.92-1.09); 0.902	<b>0.95 (0.91-1); 0.042</b>	0.96 (0.86-1.07); 0.41	1 (0.92-1.08); 0.95	0.96 (0.86-1.08); 0.48	0.54 (0.25-1.18); 0.12	<b>0.55(0.37-0.82); 0.0035</b>

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App. 13



**Post Doc  
Anniversary Evaluation - 2011/12**

**Employee Identification**

<b>Name:</b> Ganna Chornokur	<b>Employee ID:</b> 4459845
<b>Title/Rank:</b> POST DOC FELLOW	<b>Hire Date:</b> 12/30/2009
<b>Department #:</b> 25609	<b>Department:</b> Cancer Epidemiology
<b>Emp Status:</b> 50	

**PART I: BEHAVIORAL CRITERIA**

DO NOT ADJUST SECTION WEIGHT.  
SECTION WEIGHT MUST REMAIN 50%

**Behavioral Criteria**

**Score: 4.1 / 5.0 (50%)**

**Customer Focus: Commitment to Providing Outstanding Customer Service** **Score: 3.6 / 5.0 (0%)**

Conveys a positive image of Moffitt to customers. Is dedicated to superior customer service and satisfaction. Develops and maintains positive relationships with both internal and external customers. Demonstrates dignity and respect to internal and external customers.

- ☐ 4.6 – 5.0: Far Exceeds
- ☒ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**Teamwork: Work, as Part of a Group, to Achieve Results**      **Score: 4.6 / 5.0 (0%)**

Shares equally in the department's success and failures. Works effectively and constructively to find mutually beneficial solutions for all concerned parties. Shares knowledge and expertise with others to ensure the success of team and individual efforts. Supports co-workers by maintaining attendance requirements. When appropriate, works in partnership with staff, faculty and/or patient and family advisors to improve processes or care.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**Adaptability: Demonstration of Creativity, Innovation, Flexibility & Willing Acceptance of Challenge**      **Score: 4.6 / 5.0 (0%)**

Demonstrates willingness to adapt to changing individual and institutional roles, needs and environment. Exhibits a problem solving attitude; constantly seeks ways to improve processes, increase efficiency, find solutions to current situations or to develop new methods and procedures.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**Responsibility: Acting Responsibly in All Matters**      **Score: 3.6 / 5.0 (0%)**

Accepts accountability for actions, choices and outcomes; assumes nothing; answers for own conduct and obligations.

- ☐ 4.6 – 5.0: Far Exceeds
- ☒ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**Commitment to Excellence: Striving to do the Best Every Day**

**Score: 3.6 / 5.0 (0%)**

Consistently provides quality product/services. Commits to the principle of continuous improvement in the workplace. Projects pride in their work as exhibited in day-to-day interactions with staff, co-workers, patients and all those they come in contact with. Commits to the principles of patient and family-centered care: dignity & respect, information sharing, participation and collaboration.

- ☐ 4.6 – 5.0: Far Exceeds
- ☒ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**Culture of Safety: Promotion of safety & prevention of injury must be the first consideration**

**Score: 4.6 / 5.0 (0%)**

Maintains awareness and follows safety policies and procedures applicable to assigned duties. Commits to the principle of continuous improvement in the workplace. Uses sound judgment including reasonable awareness of potential hazards before acting. Promptly reports errors and/or events and/or situations of actual or potential harm.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

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**PART II: ACCOMPLISHMENTS/PROGRESS WITHIN THE PAST YEAR:**

POST DOC: In the areas below, describe your accomplishments relative to the specific area.

**Work Accomplished toward goals**

**Work**

State the project title, broad project goals and the specific progress made in the last year. If multiple projects are underway, evaluate progress with each project, identify the

relevant obstacles and include a timeline for completion.

1. MCC16701. We hypothesize, that a combination panel (DETECT) of genetic, biochemical, socio-cultural and lifestyle population-specific biomarkers and factors will provide a valuable PCa screening and risk assessment tool. The goal of this study is to evaluate a feasibility of using DETECT as a reliable approach to estimate the risk and/or detect PCa early in AAM ultimately eliminating the disparity in PCa morbidity and mortality.  
Specific progress to date.

- SRC and IRB approvals were obtained. This was actually the hardest, the longest, and the most painful process so far.
- Since active recruitment was associated with great financial, time, organizational and participation barriers, we have substituted the prospective sample collection with retrospectively collected samples in the Drs. Phelan and Park studies. The strength of this approach is that I will analyze 600 samples in total (300 African American). Thus, much greater statistical power could be achieved, compared with just 120 samples in total. In addition, Caucasian samples will also be analyzed, allowing for an interracial comparison between biomarkers.
- The data dictionary to combine the epidemiological data for the two studies has been completed.
- Sample selection and supply ordering have been completed.
- The nearest future plans: genotyping.
- The timeline for completion of the project: 09/2013, as specified by the grant.

2. MCC14648. We hypothesized that select clinical and/or bio-behavioral risk factors are associated with prostate cancer in African American men. 105 African American men aged 34-80 (35 cases and 70 controls) were recruited. Univariable and multivariable logistic regression models were built to test the marginal and joint effects of the variables when predicting the cancer status.  
Specific progress.

- The data collected during the accrual time of this protocol was analyzed and the most relevant variables were chosen for analysis.
- Statistical analysis was performed by biostatistician;
- Significant variables were identified and discussed;
- An abstract, based on the findings of this research, was submitted to the AACR 2012 meeting.
- A manuscript, based on the findings of this research, is in progress.
- Future plans: planning of a bigger study involving more African American men with prostate cancer and healthy controls.
- Timeline for completion: this secondary analysis of collected data is already completed. However, the manuscript will take another 3-6 months to be submitted.

3. MCC16112. We hypothesized that variation in the mitochondrial genome and nuclear genome-wide variation plays a role in ovarian cancer. The GOAL of this study is to more comprehensively investigate the contribution of mitochondrial genome variation to ovarian cancer risk.

Specific progress.

- The list of genes related to the pathways of cellular senescence, hormonal pathways, and chromatin remodelling have been assembled. This list yielded over 400 genes total.

- The list of SNPs related to the genes, identified within the mentioned above pathways, was assembled.
  - The selection of over 8000 of SNPs was analysed in the subset of ovarian cancer cases and controls. Several significant SNPs ( $p < 0.05$ ) in different genes have been identified.
  - Future plans: writing a manuscript and/or an abstract to disseminate our findings.
- Timeline for completion: this specific part - within one year. The project however will not end until another 4 years.

## 1. Research

1

Human Subjects (list grants number, title and IRB # in which you participated):

1. PC101913 and MCC16701 : "Comprehensive Population-Specific Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment". Funding Agency: Department of the U.S. Army (DoD) Prostate Cancer Research Program; Chornokur PI.
2. W81XWH-06-1-0034 and MCC14648: "Prostate Cancer in African American Men: Serum Biomarkers for Early Detection Using Nanoparticles". Funding Agency: Department of the U.S. Army (DoD) Prostate Cancer Research Program; Phelan PI.
3. 1R01 CA149429-01 and MCC16112: "The Mitochondrial Genome and Ovarian Cancer Risk". Funding agency: NIH; Phelan PI.

2

Vertebrate Animals (list grants number, title and IACUC # in which you participated):

N/A

## 2. Training

1

Meetings attended (w/o presentation):

1. 10/10: ASCO-NCI-EORTC Annual Meeting on Molecular Markers in Cancer, Florida
2. Center for Equal Health/ P20 Strategic Planning Retreat Meeting; date: 09/29/2011



2

Workshops/Training completed in the last year (Research, Ethics, Human Subjects, Biosafety, Vertebrate Animals use-include date completed):

IRB and Bioethics completed ~2 years ago  
Mandatory Education test: 05/2011

3

Optional Workshop/Training completed in last year (Communication Skills, Clinical Trials, Grant/Scientific Writing, technical Workshops, English as a Second Language):

EndNote training at USF library: approx date 12/2010;  
Clinical trials, Grant/Scientific Writing completed ~2 years ago;

4

Other Training:

Cancer Epidemiology course: In progress;  
Cancer Health Disparities course: In progress;  
Biostats 101 lecture series: 10-11 2011 (Certificate of Completion Awarded);  
01/11: 11-hour educational course, entitled "Cultural and Linguistic Competency: A 21st Century Response to Historical & Unending Inequities" (Certificate of Completion Awarded);  
09/23/2011: "Feed your future", 3-hour lecture series in recognition of Infant Mortality Month at USF.

### 3. Publications/Presentations

1

Peer-reviewed publications:

1. Shahnjaya Connors, Ganna Chornokur, Nagi Kumar. New Insights to the Mechanisms of Green Tea Catechins in the Chemoprevention of Prostate Cancer. Accepted to the International Journal of Nutrition and Cancer, 2011
2. G. Chornokur, Kyle Dalton, M. Borysova, and N. Kumar. Disparities at Presentation, Diagnosis, Treatment and Survival in African American Men, Affected by Prostate Cancer. "The Prostate", 71(9), 985-97, 2011
3. G. Chornokur, S. Arya, C. Phelan, R. Tanner and S. Bhansali. Impedance-based Miniaturized Biosensor for Ultrasensitive and Fast Prostate Specific Antigen Detection. Hindawi "Journal of Sensors", vol 2011, Article ID 983752, 7 pages
4. Manju Venugopal, Sunil K. Arya, Ganna Chornokur, Shekhar Bhansali. A Realtime and Continuous Assessment of Cortisol in ISF Using Electrochemical

## Impedance Spectroscopy // Sensors and Actuators A, 2011

5. Sunil K. Arya, Ganna Chornokur, Manju Venugopal and Shekhar Bhansali. Antibody functionalized Interdigitated -electrode (ID E) based Impedimetric cortisol biosensor // Analyst, 2010, 135, 1941 - 1946

6. S. Arya; G. Chornokur, M. Venugopal; and S. Bhansali. Dithiobis(succinimidyl propionate) Modified Gold Micro Array Electrode Based Electrochemical Immunosensor for Ultrasensitive Detection of Cortisol // Biosensors and Bioelectronics 2010 25(10), 2296-2301

## 2

Other (non peer-reviewed) publications:

N/A

## 3

Manuscripts that are in preparation or under review:

1. Ganna Chornokur and Catherine M Phelan. Global ovarian cancer disparities: a review.

2. Catherine M. Phelan, Robert Royer, Shiyu Zhang, Song Li, Kelly Metcalfe, Aletta Poll, Ping Sun, Ganna Chornokur, Steven A. Narod. Risks of gastrointestinal cancers in female BRCA1 and BRCA2 mutation carriers.

3. Ganna Chornokur, Gang Han, Richard Tanner, Julio Pow-Sang, and Catherine Phelan. Biomarkers of prostate cancer in African American men.

4. Meghan E. Borysova, Ganna Chornokur, Kyle J. Dalton, and Dawood H. Sultan. Prostate Cancer Health Disparities throughout the Cancer Control Continuum.

## 4

Abstracts/Posters/Talks presented (Indicate authors, title, date, venue):

06/2011, Orlando, FL: Joining FORCES Against Hereditary Cancer Conference – presenter. The poster presentation entitled “Risk of Gastrointestinal Cancers in Female BRCA1 and BRCA2 Mutation Carriers”.

03/2011, Tampa, FL: The Moffitt Research Institute Scientific Retreat Conference – presenter. The poster presentation entitled “Comprehensive Population-Specific Bio-Behavioral Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment: a DETECT study”.

10/2010, Miami, FL: 3rd AACR conference “The Science of Health Disparities” – presenter. The poster presentation entitled “Comprehensive Population-Specific Marker Panel for Prostate Cancer Screening”.

Abstract submitted for poster presentation (acceptance status is yet unknown):

Submission date 11/2011. Conference: Chicago, IL 03/2012: Annual AACR meeting - presenter. The abstract entitled "Risk Factors of Prostate Cancer in African American Men".

#### 4. Honors and Awards

1

Open Comment:

N/A

#### 5. Grants/Proposals

Include PI, Agency, Date, Total Cost

1

Proposals funded:

11/2010: The recipient (Principal Investigator) of the 2-year Health Disparity Training Award: "Comprehensive Population-Specific Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment". Funding Agency: Department of the U.S. Army (DoD) Prostate Cancer Research Program; Start Date: 08/2011.  
Total cost: ~115,000

2

Proposals submitted:

N/A

3

Proposals In preparation/planning:

Chemopreventive Effects of Curcumin in Prostate Cancer Cell Lines, Derived from African American and European American Men: R03 proposal in preparation.

#### 6. Teaching (if applicable)

1

List teaching responsibilities. Include individuals mentored: student name, program, dates:

I am currently mentoring a USF PhD student, Edikan Archibong. Edikan's primary department is Biomedical and Chemical Engineering and she's pursuing research in biosensors in cancer. As she has just started in August 2011, her final topic is still in preparation. However, she is learning cancer biology in general and methods (including ELISA) in particular, and I am her immediate mentor in this process. Edikan will be conducting research in Dr. Phelan/Alvaro's labs at Moffitt, as well as at the Engineering department at USF.

## 7. Service and Administration (if applicable)

1

National level:

N/A

2

Local (Moffitt/USF) level:

N/A

3

Research group or lab level:

N/A

## 8. Career Progress

1

Career goals:

My long-term career goal is to contribute to the resolution of cancer-related health disparities by conducting innovative, high-impact research as an independent investigator with competitive, peer-reviewed funding.

This broad, long-term goal is being achieved through methodologically

organized series of the following structured activities:

1. Structured mentoring program coordinated by the mentoring team. This includes weekly meetings with Dr. Phelan; bi-weekly meetings with Dr. Kumar; meetings with Dr. Park and biostatisticians on as-needed basis.
2. Become Familiar with the Major Studies and Findings in the Area of cancer disparity. This is achieved by regular attendance of relevant Moffitt ground rounds and USF lecture series; systematic review of the cutting edge literature in cancer health disparities field; and attendance of relevant conferences, seminars and classes.
3. Gain Expertise in Methodologies Needed to Conduct Transdisciplinary cancer disparity research. This is achieved by gaining experience in the cutting edge laboratory (i.e. genotyping) as well as bio-behavioral (i.e. community participatory research) techniques.
4. Be Able to Critically Review and Evaluate Research in Cancer disparity. This is achieved through attendance of journal clubs, as well as through discussions with my Mentoring team and writing my own review manuscripts that were published and are getting ready for submission to high impact peer-reviewed journals.
5. Gain an Understanding of Fundamental Issues Regarding the Ethical Conduct of Research, and responsible conduct of human research. This was achieved through attending Bioethics for researchers class (certificate awarded), as well as IRB certification (certificate awarded), and by discussions with my Mentors who are engaged in clinical trials and other human subjects' research.
6. Attend and present at relevant research and educational meetings. Please see the previous sections for details on the meetings and presentations.
7. Scientific writing and research dissemination. Please see the previous sections for details on the published manuscripts and manuscripts in preparation.
8. Applying for peer-reviewed research funding. A training grant was funded by the DoD; in addition, an R03 submission is in preparation. Please see the details in the previous sections.
9. Contribute to raising a new generation of students interested in cancer research. This is achieved by mentoring a PhD student. Please see details in the previous section.

---

## 2

How are you making necessary connections:

1. Regular attendance of Moffitt ground rounds, including designated meeting with the speaker (only in case of the most relevant topics / similar research interests);
2. Regular attendance of relevant seminars and lectures given at USF, including meeting with the speaker (only in case of the most relevant topics / similar research interests);
3. Attending and presenting at the high impact conferences, including the AACR and ASCO meetings;
4. Being introduced to the Moffitt scientists/members by my Mentor(s);
5. Active collaboration with several Moffitt cores, including biostatistics and survey cores.

3

List jobs applied to or Interviews which have taken place (Optional):

N/A.

I have started the execution of my funded 2-year proposal just a few months ago. I consider successful completion of this proposal to be an important step in my career, and since Moffitt is an ideal environment for this purpose, I am NOT looking for other jobs at this time.

4

Other career oriented accomplishments/concerns:

N/A

### PART III: NUMERICAL EVALUATION

EVALUATOR: Assign a score to each area (see rating scale below).  
Weights on areas may be adjusted but must be whole numbers only.  
Weights of 8 areas must add up to 50%

#### Rating Scale

**4.6 – 5.0: Far Exceeds-** Outstanding performance that always exceeds expectations and is demonstrated for an extended period of time.

**3.6 – 4.5: Surpasses -** Very strong performance that exceeds expectations in most situations and meets expectations in all others.

**2.5 – 3.5: Successfully Meets-** Consistently meets performance expectations.

**1.0 – 2.4: Does Not Meet -** Indicates inconsistent performance, meeting some, but not all skill requirements. Immediate and substantial improvements must be made.

Work Accomplished  
towards goals

Score: 4.6 / 5.0 (7%)

Score 1-5. If not applicable, change weight to zero

Score: 4.6 / 5.0 (0%)

Training

Score: 4.5 / 5.0 (7%)

Score 1-5. If not applicable, change weight to zero

Score: 4.5 / 5.0 (0%)

**Publications** **Score: 4.6 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 4.6 / 5.0 (0%)**

**Honors and Awards** **Score: 4.0 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 4.0 / 5.0 (0%)**

**Grants/Proposals** **Score: 4.5 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 4.5 / 5.0 (0%)**

**Teaching  
(if applicable)** **Score: 4.0 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 4.0 / 5.0 (0%)**

**Service and  
Administration  
(if applicable)** **Score: 3.6 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 3.6 / 5.0 (0%)**

**Career Progress** **Score: 4.6 / 5.0 (6%)**

**Score 1-5. If not applicable, change weight to zero** **Score: 4.6 / 5.0 (0%)**

**Summary Score** **Score: 4.3 / 5.0 (50%)**

Summary of Numerical Evaluation area. Area weight should equal 50%

## **Numerical Evaluation Comments**

**Evaluator comments related to the numerical evaluation:**

**OVERALL SCORE (FINAL SCORE)**      **Score: 4.2 / 5.0 (100%)**

The Overall Score is made up of the Numerical Evaluation and Behavioral Competencies areas

## **PART IV: NEW GOALS**

POST DOC: Propose goals and expectations for the next year. Identify potential obstacles and propose solutions.

EVALUATOR: Populate the Approved Goals areas as applicable, using the Proposed Goals as a guide.

### **Post Doc's Proposed Goals: Research/Scholarship**

---

Main focus: Continue the execution of MCC16701 (DoD DETECT training grant); aim 1 should be completed and analysed and aim 2 should be initiated.

Also, continue the MCC16112 and MCC14648 as it related to data analysis and research dissemination.

### **Approved Goals: Research/Scholarship**

---

Main focus: Continue the execution of MCC16701 (DoD DETECT training grant); aim 1 should be completed and analysed and aim 2 should be initiated.

Also, continue the MCC16112 and MCC14648 as it related to data analysis and research dissemination.

### **Post Doc's Proposed Goals: Training**

---

- Continue regular attendance of relevant workshops, Moffitt Ground rounds and USF lectures;



- Audit the following classes: Cancer biology III; tentative: health disparities (subject to availability);
- Clinical: shadow research coordinators from Dr. Kumar's group as they recruit patients into the clinical trials;
- Tentative: phlebotomy course;
- Tentative: grant writing workshop(s) (subject to availability).
- Tentative: shadow an MCC prostate cancer physician (subject to availability).

### **Approved Goals: Training**

---

- Continue regular attendance of relevant workshops, Moffitt Ground rounds and USF lectures;
- Audit the following classes: Cancer biology III; tentative: health disparities (subject to availability);
- Clinical: shadow research coordinators from Dr. Kumar's group as they recruit patients into the clinical trials;
- Tentative: phlebotomy course;
- Tentative: grant writing workshop(s) (subject to availability).
- Tentative: shadow an MCC prostate cancer physician (subject to availability).

### **Post Doc's Proposed Goals: Publications/Presentation**

---

Publications: at least two 1-author publications in the relevant peer-review journals accepted for publication.

Presentations: at least two poster and/or oral presentations at the high-impact scientific meetings / conferences. Possible meetings may include AACR meeting 03/2012 (abstract submitted), AACR Cancer health disparity meeting 09-10/2012, relevant prostate cancer meetings and/or other high impact health disparity meetings to be held in 2012.

### **Approved Goals: Publications/Presentation**

---

Publications: at least two 1-author publications in the relevant peer-review journals accepted for publication.

Presentations: at least two poster and/or oral presentations at the high-impact scientific meetings / conferences. Possible meetings may include AACR meeting 03/2012 (abstract submitted), AACR Cancer health disparity meeting 09-10/2012, relevant prostate cancer meetings and/or other high impact health disparity meetings to be held in 2012.

### **Post Doc's Proposed Goals: Honors and Awards**

---

AACR Scholar-in-Training Awards: Available to early-career scientists presenting a meritorious proffered paper at the AACR Annual Meeting.  
I have applied for this award at the time of abstract submission.

---

**Approved Goals: Honors and Awards**

---

AACR Scholar-in-Training Awards: Available to early-career scientists presenting a meritorious proffered paper at the AACR Annual Meeting.  
I have applied for this award at the time of abstract submission.

---

**Post Doc's Proposed Goals: Grants/Proposals**

---

At least one NIH R03 proposal submitted and/or  
at least one DoD proposal submitted (other than the training grant).  
Focus: prostate cancer health disparities

---

**Approved Goals: Grants/Proposals**

---

At least one NIH R03 proposal submitted and/or  
at least one DoD proposal submitted (other than the training grant).  
Focus: prostate cancer health disparities

---

**Post Doc's Proposed Goals: Teaching**

---

Continue mentoring Edikan Archibong.  
Tentative: mentoring other students, including Summer Interns like SPARK/LINK.

---

**Approved Goals: Teaching**

---

Continue mentoring Edikan Archibong.  
Tentative: mentoring other students, including Summer Interns like SPARK/LINK.

## **Post Doc's Proposed Goals: Service and Administration**

---

At this point, I don't have any goals in this area.

## **Approved Goals: Service and Administration**

---

At this point, I don't have any goals in this area.

## **Post Doc's Proposed Goals: Career**

---

To continue my transition to an independent researcher by the means of:

1. Enhancing my research skills:
  - a. Molecular methods
  - b. Biobehavioral components
  - c. Learning about Cancer disparities
  - d. Writing publications
  - e. Presenting my research
2. Gaining career development skills:
  - a. Grant submissions (R03, DoD idea development)
  - b. Forming research collaborations
  - c. Management of the limited funds, available through the DoD training grant, and budgeting

## **Approved Goals: Career**

---

To continue my transition to an independent researcher by the means of:

1. Enhancing my research skills:
  - a. Molecular methods
  - b. Biobehavioral components
  - c. Learning about Cancer disparities
  - d. Writing publications
  - e. Presenting my research
2. Gaining career development skills:
  - a. Grant submissions (R03, DoD idea development)
  - b. Forming research collaborations
  - c. Management of the limited funds, available through the DoD training grant, and

budgeting

**New Goals Overall Comments (Optional):**

**Evaluator may use this area to make comments related to new goals:**

Overall, Ganna is doing very well. We need to start Ganna's entrance into service and administration and increase her teaching.

**Post Doc Final Comments (optional):**

**Evaluator Final Comments (optional):**

Employee:

Ganna Chornokur G.C. (electronic signature for the evaluation of  
Ganna Chornokur)

Date:

01/03/2012 09:15 AM

Manager: Catherine Phelan C.P. (electronic signature for the evaluation of  
Ganna Chornokur)

Date: 01/03/2012 10:08 AM

App. 14



**Post Doc  
Anniversary Evaluation - 2012/13**

**Employee Identification**

**Name:** Ganna Chornokur  
**Title/Rank:** POST DOC FELLOW  
**Department #:** 25609  
**Emp Status:** 50

**Employee ID:** 4459845  
**Hire Date:** 12/30/2009  
**Department:** Cancer Epidemiology

**Instructions**

The Post Doc will start off the evaluation by completing Parts II and IV only. The evaluator will then complete the remaining sections.

**PART I: BEHAVIORAL CRITERIA**

DO NOT ADJUST SECTION WEIGHT.  
SECTION WEIGHT MUST REMAIN 50%

**Behavioral Criteria**

**Score: 4.6 / 5.0  
(50%)**

**Customer Focus: Commitment to Providing Outstanding Customer Service      Score: 4.6 / 5.0 (0%)**

Conveys a positive image of Moffitt to customers. Is dedicated to superior customer service and satisfaction. Develops and maintains positive relationships with both internal and external customers. Demonstrates dignity and respect to internal and external customers.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets

☐ 1.0 – 2.4: Does Not Meet

**Comments:**

---

**Teamwork: Work, as Part of a Group, to Achieve Results**      **Score: 4.6 / 5.0 (0%)**

Shares equally in the department's success and failures. Works effectively and constructively to find mutually beneficial solutions for all concerned parties. Shares knowledge and expertise with others to ensure the success of team and individual efforts. Supports co-workers by maintaining attendance requirements. When appropriate, works in partnership with staff, faculty and/or patient and family advisors to improve processes or care.

- ☒ 4.6 – 5.0: Far Exceeds  
☐ 3.6 – 4.5: Surpasses  
☐ 2.5 – 3.5: Successfully Meets  
☐ 1.0 – 2.4: Does Not Meet

**Comments:**

---

**Adaptability: Demonstration of Creativity, Innovation, Flexibility & Willing Acceptance of Challenge**      **Score: 4.6 / 5.0 (0%)**

Demonstrates willingness to adapt to changing individual and institutional roles, needs and environment. Exhibits a problem solving attitude; constantly seeks ways to improve processes, increase efficiency, find solutions to current situations or to develop new methods and procedures.

- ☒ 4.6 – 5.0: Far Exceeds  
☐ 3.6 – 4.5: Surpasses  
☐ 2.5 – 3.5: Successfully Meets  
☐ 1.0 – 2.4: Does Not Meet

**Comments:**

---

**Responsibility: Acting Responsibly in All Matters**      **Score: 4.6 / 5.0 (0%)**

Accepts accountability for actions, choices and outcomes; assumes nothing; answers for own conduct and obligations.

- ☒ 4.6 – 5.0: Far Exceeds  
☐ 3.6 – 4.5: Surpasses  
☐ 2.5 – 3.5: Successfully Meets  
☐



1.0 – 2.4: Does Not Meet

**Comments:**

---

**Commitment to Excellence: Striving to do the Best Every Day**

**Score: 4.6 / 5.0 (0%)**

Consistently provides quality product/services. Commits to the principle of continuous improvement in the workplace. Projects pride in their work as exhibited in day-to-day interactions with staff, co-workers, patients and all those they come in contact with. Commits to the principles of patient and family-centered care: dignity & respect, information sharing, participation and collaboration.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

---

**Culture of Safety: Promotion of safety & prevention of injury must be the first consideration**

**Score: 4.6 / 5.0 (0%)**

Maintains awareness and follows safety policies and procedures applicable to assigned duties. Commits to the principle of continuous improvement in the workplace. Uses sound judgment including reasonable awareness of potential hazards before acting. Promptly reports errors and/or events and/or situations of actual or potential harm.

- ☒ 4.6 – 5.0: Far Exceeds
- ☐ 3.6 – 4.5: Surpasses
- ☐ 2.5 – 3.5: Successfully Meets
- ☐ 1.0 – 2.4: Does Not Meet

**Comments:**

**PART II: ACCOMPLISHMENTS/PROGRESS WITHIN THE PAST YEAR**

POST DOC: In the areas below, describe your accomplishments relative to the specific subject.

**If you do not see any goals from last year listed in the sections below and you participated in last year's evaluation process, please reference last year's appraisal saved in the "My Performance" section of your dashboard in Halogen.**

**If you are a new Post Doc, you will not have any pre-defined Goals.**

## Work Accomplished toward goals

### Work

State the project title, broad project goals and the specific progress made in the last year. If multiple projects are underway, evaluate progress with each project, identify the relevant obstacles and include a timeline for completion.

1. MCC16701: We hypothesize, that a combination panel (DETECT) of genetic, biochemical, socio-cultural and lifestyle population-specific biomarkers and factors will provide a valuable PCa screening and risk assessment tool.

Specific progress made in the last year.

1. Genotyping for the select SNPs of interest have been successfully completed in Black and White DNA samples.

2. Statistical analysis have been completed.

3. We have found that in African American (but not European American) men there is interaction between prostate cancer risk, obesity and select germline SNPs.

4. These data were presented as a poster at the AACR health disparities conference and will be presented at the NIH health disparities summit in December 2012.

5. The manuscript is in preparation.

6. Using these data as preliminary, an R21 have been submitted in November 2012, entitled "Obesity and prostate cancer risk in Black and White men: a functional approach".

7. An annual DoD report was completed and accepted by the DoD as written.

8. My Mentoring team and I have decided to proceed with genotyping the CAG repeat region in the AR gene in the same germline samples.

9. Genotyping has now been completed and data analysis is in progress.

2. MCC14648: We hypothesized that select clinical and/or bio-behavioral risk factors are associated with prostate cancer in African American men. 105

African American men aged 34-80 (35 cases and 70 controls) were recruited. Univariable and multivariable logistic regression models were built to test the marginal and joint effects of the variables when predicting the cancer status.

Specific progress within the last year:

1. Data analysis was completed.

2. A poster presentation, entitled "Risk factors for prostate cancer in African American men" was presented by me at the Annual AACR meeting 2012.

3. The manuscript entitled "High Grade Prostate Intraepithelial Neoplasia (PIN) is a PSA-independent risk factor for prostate cancer in African American men: a pilot study" by Ganna Chornokur; Gang Han; Richard Tanner; Hui-Yi Lin; Lee B Green, Julio Pow-Sang; Catherine M Phelan was completed and submitted to "Cancer Letters".

4. The Journal was potentially interested in publication, but some edits were requested. Hence, a revised version was submitted 11/2012. Given favorable peer-review, we expect that the manuscript will be accepted.

5. Using these data as preliminary, a grant project entitled "Prostatic Intraepithelial Neoplasia and prostate cancer in African American men" was submitted to the DoD 08/2012. Dr. Catherine Phelan is a PI and I am a co-I on this project.

3. MCC16112: We hypothesized that variation in the mitochondrial genome and nuclear genome-wide variation plays a role in ovarian cancer. The goal of this study is to more comprehensively investigate the contribution of mitochondrial genome variation to ovarian cancer risk.

Specific progress made in the last year:

1. Genotyping have been completed. Eight key cancer-relevant pathways were selected. I am responsible for analysis of the transmembrane and metabolism pathway that includes 305 SNPs from 120 genes.

2. Using these data, an abstract entitled "Variation in the cellular transport genes influence epithelial ovarian cancer risk and histopathologic subtype" was submitted to the AACR Annual Meeting 2013. I am a presenter and Dr Phelan is a senior author.

3. A manuscript entitled "Variation in the cellular transport genes influence epithelial ovarian cancer risk and histopathologic subtype" is in progress.

4. A review manuscript entitled "Global ovarian cancer health disparities" has been submitted to Gynecologic Oncology Journal. The manuscript was accepted pending minor revisions. A revised version is expected to be submitted to the journal next week. Prompt approval is anticipated.

## Existing Research Goals

---

Main focus: Continue the execution of MCC16701 (DoD DETECT training grant); aim 1 should be completed and analysed and aim 2 should be initiated.

Also, continue the MCC16112 and MCC14648 as it related to data analysis and research dissemination.

## Research

### 1

Human Subjects (list grants number, title and IRB # in which you participated):

1. MCC 14648: Nanoscale Detection of Plasma Prostate Cancer Cells in African American Men; IRB#: Pro00001823 (Phelan PI)

2. MCC 16701: Comprehensive Population-Specific Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment; IRB#: Pro00004885 (Chornokur PI)

3. MCC 16112: The Mitochondrial Genome and Ovarian Cancer Risk; IRB#: Pro00000249 (Phelan PI)

---

### 2

Vertebrate Animals (list grants number, title and IACUC # in which you participated):

N/A

## Existing Training Goals

---

- Continue regular attendance of relevant workshops, Moffitt Ground rounds and USF lectures;
- Audit the following classes: Cancer biology III; tentative: health disparities (subject to availability);
- Clinical: shadow research coordinators from Dr. Kumar's group as they recruit patients into the clinical trials;
- Tentative: phlebotomy course;
- Tentative: grant writing workshop(s) (subject to availability).
- Tentative: shadow an MCC prostate cancer physician (subject to availability).

## Training

### 1

Meetings attended (w/o presentation):

N/A

---

### 2

Workshops/Training completed in the last year (Research, Ethics, Human Subjects, Biosafety, Vertebrate Animals use-include date completed):

1. Moffitt Annual Mandatory Continuing Education Test: 05/2012
  2. IRB bi-annual test: 04/2012
  3. Biosafety annual continuing education module: 04/2012
- 

### 3

Optional Workshop/Training completed in last year (Communication Skills, Clinical Trials, Grant/Scientific Writing, technical Workshops, English as a Second Language):

1. Interactive NIH Grant Writing workshop held during the Annual AACR Meeting: 03/2012
- 

### 4

Other Training:

1. Moffitt Office Of Technology and Commercialization (OTMC) internship: 10/2012 - 08/2013 (in progress).
2. USF postdoctoral training workshop, entitled "Negotiate offers for faculty positions": 09/2012.

## Existing Publication/Presentation Goals

---

**Publications:** at least two 1-author publications in the relevant peer-review journals accepted for publication.

**Presentations:** at least two poster and/or oral presentations at the high-impact scientific meetings / conferences. Possible meetings may include AACR meeting 03/2012 (abstract submitted), AACR Cancer health disparity meeting 09-10/2012, relevant prostate cancer meetings and/or other high impact health disparity meetings to be held in 2012.

### Publications/Presentations

#### 1

Peer-reviewed publications:

1. Kryshtab TG, Borkovska LV, Kolomys O, Korsunska NO, Strelchuk VV, Germash LP, Pechers'ka KYu, Chornokur G, Ostapenko SS, Phelan CM, Stroyuk OL. The effect of bio-conjugation on aging of the photoluminescence in CdSeTe-ZnS core-shell quantum dots. Superlattice Microst. 2012;51:353-62. DOI: 10.1016/j.spmi.2011.12.005
2. Kumar NB, Crocker T, Smith T, Pow-Sang J, Spiess PE, Connors SK, Chornokur G, Dickinson IS, Williams CR, Salup R, Bai W. Prostate Cancer Chemoprevention targeting High Risk Populations: Model for trial design and outcome measures. J Cancer Sci Ther. 2012 ; 2011(S3). PMID: 22422102
3. Connors S, Chornokur G, Kumar N. New Insights to the Mechanisms of Green Tea Catechins in the Chemoprevention of Prostate Cancer. Nutr Cancer. 2012;64(1): 4-22. PMID: 22098273

---

#### 2

Other (non peer-reviewed) publications:

N/A

---

#### 3

Manuscripts that are in preparation or under review:

1. Chornokur G, Amankwah E, Schildkraut JM, Phelan CM. Global ovarian cancer health disparities. Submitted to Gynecologic Oncology [accepted pending minor revisions]
2. Chornokur G and Kumar NB. Evidence-based strategies for prostate cancer chemoprevention in men of African descent. Submitted to Cancer Causes and Control [major revision requested]

3. Chornokur G; Han G; Tanner R; Lin H-Y; Green LB, Pow-Sang J; Phelan CM. High Grade Prostate Intraepithelial Neoplasia (PIN) is a PSA-independent risk factor for prostate cancer in African American men. Submitted to Cancer Letters [accepted pending revisions]
  4. Chornokur G, Amankwah E, Phelan CM, Park JY, Pow-Sang J, Kumar NB. Variation in the metabolism related genes and obesity may contribute to prostate cancer disparity in African American men. In preparation
  5. Ganna Chornokur, Jonathan Tyrer, Hui-Yi Lin, Gang Han, Xiaotao Qu, Zhihua Chen, Ya-Yu Tsai, Ellen L. Goode, Julie M. Cunningham, Edwin Iversen, Susan Ramus, Andrew Berchuck, Joellen Schildkraut, Alvaro Monteiro, Simon Gayther, Steven A. Narod, Paul Pharoah, Thomas A. Sellers and Catherine M. Phelan. Variation in the cellular transport genes influence epithelial ovarian cancer risk and histopathologic subtype. In preparation.
  6. Kumar NB, Chornokur G, Dalton K et al. Comparing the watchful waiting and radical prostatectomy cohorts in a comprehensive NIH-designated cancer center with uniform assignment criteria. In preparation.
- 

#### **4**

Abstracts/Posters/Talks presented (Indicate authors, title, date, venue):

1. AACR Annual Meeting (03-04, 2012): Risk Factors of Prostate Cancer in African American Men (poster presentation): presenter
2. AACR "The Science of Health Disparities" Conference (10, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation): presenter
3. NIH Summit to Reduce Health Disparities (12, 2012): Polymorphisms in the metabolism related genes and obesity may explain prostate cancer disparity in African American men (poster presentation): presenter
4. AACR Annual Meeting (04, 2013): Variation in the cellular transport genes influence epithelial ovarian cancer risk and histopathologic subtype (abstract submitted, presenter)

### **Existing Honors and Awards**

---

AACR Scholar-in-Training Awards: Available to early-career scientists presenting a meritorious proffered paper at the AACR Annual Meeting.  
I have applied for this award at the time of abstract submission.

### **Honors and Awards**

**1**

Open Comment:

1. Center for Equal Health (CEH) travel award to attend and present at the 2012 Annual AACR meeting (Chicago, IL), 04, 2012 [\$500]
2. Scholar-in-training Award to attend the Fifth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, 10, 2012 [\$1250]

## **Existing Grants/Proposals**

---

At least one NIH R03 proposal submitted and/or  
at least one DoD proposal submitted (other than the training grant).  
Focus: prostate cancer health disparities

### **Grants/Proposals**

include PI, Agency, Date, Total Cost

**1**

Proposals funded:

1. Department of the U.S. Army (DoD) Prostate Cancer Research Program, Health Disparity Training Award: Comprehensive Population-Specific Marker Panel for Early Prostate Cancer Diagnostics and Risk Assessment, 08/2011 – 07/2013 [funded and active]

---

**2**

Proposals submitted:

1. Department of the U.S. Army (DoD) Prostate Cancer Research Program, Hypothesis Development Award: Comparative race-specific chemopreventive effects of curcumin in prostate cancer, 06/2012 [undergoing peer-review]
  2. National Institute of Health (NIH) Exploratory/Developmental Grants Program for Basic Cancer Research in Cancer Health Disparities (R21): Obesity, tumor biology, genetics and prostate cancer risk in men of African and European descent, 11/2012 [undergoing peer-review]
  3. National Institute of Health (NIH) Cancer Prevention Research Small Grant Program (R03): Comparative race-specific chemopreventive effects of curcumin in prostate cancer, 02/2012 [not funded]
-

**3**

Proposals in preparation/planning:

1. Department of the U.S. Army (DoD) Prostate Cancer Research Program, Health Disparity Research Award. Due date 08/2013. Proposal is in the planning stage, final topic dependent on the results of my current experimental work (genotyping and analysis of the CAG repeat in the AR gene in Black and White men with prostate cancer and controls).

### **Existing Teaching Goals (if applicable)**

---

Continue mentoring Edikan Archibong.

Tentative: mentoring other students, including Summer interns like SPARK/LINK.

### **Teaching (if applicable)**

**1**

List teaching responsibilities. Include individuals mentored: student name, program, dates:

Edikan Archibong has changed her research direction. However, I helped Dr Phelan supervise an INSPIRE (Interest, Need, Stimulate, Persevere, Ideas, Relevance and Excellence) Undergraduate Summer Student (Jennifer Diaz), 06-09/ 2012.

- Project entitled "Breast Cancer in Latin America"

### **Existing Service and Administration Goals (if applicable)**

---

At this point, I don't have any goals in this area.

### **Service and Administration (if applicable)**

**1**

National level:

N/A

---

**2**

Local (Moffitt/USF) level:



N/A

---

**3**

Research group or lab level:

N/A

## **Existing Career Progress Goals**

---

To continue my transition to an independent researcher by the means of:

1. Enhancing my research skills:

- a. Molecular methods
- b. Biobehavioral components
- c. Learning about Cancer disparities
- d. Writing publications
- e. Presenting my research

2. Gaining career development skills:

- a. Grant submissions (R03, DoD idea development)
- b. Forming research collaborations
- c. Management of the limited funds, available through the DoD training grant, and budgeting

## **Career Progress**

**1**

Career goals:

- 1. Grant submissions: at least one new submission and/or re-submission as a P.I. However, my goal is at least two submissions a year (including new submissions and revisions). I have completed 3 new grant submissions during the last year.
- 2. Peer-reviewed publications: at least two relevant peer-reviewed publications accepted a year, preferably at least one of them 1st author. While I had 3 peer-reviewed publications last year, none of them is 1st author. However, at present I have 3 1st author publications favorably peer-reviewed. It is expected that at least 2 of them will be promptly accepted following minor revisions.
- 3. Presentations: at least two relevant presentations at the high-impact scientific meetings. Last year, I had three conference poster presentations and was awarded two travel grants to attend the conferences. The goal for the next year is to continue this schedule, with an emphasis on oral presentations.
- 4. Training: I am continuously attending the relevant scientific ground rounds

and seminars at Moffitt and USF at the average of 1 per week. I am keeping my IRB, Biosafety and other mandatory trainings and certifications up to date. In addition, I am doing an internship at the OTMC. Although not directly relevant to my current work, this is a great opportunity to broaden my horizons and provide me with an advance in the job search.

5. Job search: I am currently on the road to independence, and looking for suitable academic faculty positions. So far, I have applied to one highly relevant position at the MD Anderson. I have several other open job positions in mind which I may apply later on. In addition, I am continuously looking for new job opportunities via a variety of sources, including Internet, conferences, networking etc.

6. Networking. I am using the conferences as main networking opportunities. In addition, I am attending the "Expert lunches" with the Invited Ground Rounds speakers (where possible).

---

## 2

How are you making necessary connections:

Not sure what this means exactly, but I am utilizing all the steps specified in the previous bullet (1. Career goals) to achieve career progress.

---

## 3

List jobs applied to or interviews which have taken place (Optional):

1. Duncan Family Institute Mentored Junior Faculty Fellowship in Cancer Prevention at the MD Anderson Cancer Center: applied 11/2012.

---

## 4

Other career oriented accomplishments/concerns:

If possible, I would like to get involved into service and administration at any level.

## PART III: NUMERICAL EVALUATION

Assign a score to each area (see rating scale below).

Weights on areas may be adjusted but must be in whole numbers only.

Weights of 8 areas must add up to 50%

### Rating Scale

**4.6 – 5.0: Far Exceeds**- Outstanding performance that always exceeds expectations and is demonstrated for an extended period of time.

**3.6 – 4.5: Surpasses** - Very strong performance that exceeds expectations in most situations and meets expectations in all others.

**2.5 – 3.5: Successfully Meets-** Consistently meets performance expectations.

**1.0 – 2.4: Does Not Meet** - Indicates inconsistent performance, meeting some, but not all skill requirements. Immediate and substantial improvements must be made.

Work Accomplished towards goals	Score: 4.5 / 5.0 (7%)
Score 1-5. If not applicable, change weight to zero	Score: 4.5 / 5.0 (0%)
Training	Score: 4.5 / 5.0 (7%)
Score 1-5. If not applicable, change weight to zero	Score: 4.5 / 5.0 (0%)
Publications	Score: 4.3 / 5.0 (6%)
Score 1-5. If not applicable, change weight to zero	Score: 4.3 / 5.0 (0%)
Honors and Awards	Score: 4.0 / 5.0 (6%)
Score 1-5. If not applicable, change weight to zero	Score: 4.0 / 5.0 (0%)
Grants/Proposals	Score: 4.0 / 5.0 (6%)
Score 1-5. If not applicable, change weight to zero	Score: 4.0 / 5.0 (0%)
Teaching (if applicable)	Score: 4.3 / 5.0 (6%)

**Score 1-5. If not applicable, change weight to zero**

**Score: 4.3 / 5.0 (0%)**

**Service and Administration  
(if applicable)**

**Score: 4.3 / 5.0  
(6%)**

**Score 1-5. If not applicable, change weight to zero**

**Score: 4.3 / 5.0 (0%)**

**Career Progress**

**Score: 4.0 / 5.0  
(6%)**

**Score 1-5. If not applicable, change weight to zero**

**Score: 4.0 / 5.0 (0%)**

**Summary Score**

**Score: 4.2 / 5.0 (50%)**

Summary of Numerical Evaluation area. Area weight should equal 50%

### **Numerical Evaluation Comments**

**Evaluator comments related to the numerical evaluation:**

**OVERALL SCORE (FINAL SCORE)**

**Score: 4.4 / 5.0 (100%)**

The Overall Score is made up of the Numerical Evaluation and Behavioral Competencies areas

### **PART IV: NEW GOALS**

POST DOC: Propose goals and expectations for the next year. Identify potential obstacles and propose solutions.

EVALUATOR: Populate the Approved Goals areas as applicable, using the Proposed Goals as a guide.

### **Post Doc's Proposed Goals: Research/Scholarship**

- 
1. Continue and finish the Aim 2 of the DoD training award. Successfully complete the grant, write the final evaluation etc.
  2. Continue working on the ovarian mitochondrial genome project. Conduct some

functional work to elucidate the biological significance of the identified SNPs and genes on ovarian carcinogenesis.

### **Approved Goals: Research/Scholarship**

---

1. Continue and finish the Aim 2 of the DoD training award. Successfully complete the grant, write the final evaluation etc.
2. Continue working on the ovarian mitochondrial genome project. Conduct some functional work to elucidate the biological significance of the identified SNPs and genes on ovarian carcinogenesis.

### **Post Doc's Proposed Goals: Training**

---

1. Finish the OTMC internship
2. Audit the Cancer genetics course (Dr Alvaro Monteiro)
3. Continuous attending of the relevant scientific ground rounds, seminars, talks etc at Moffitt and/or USF

### **Approved Goals: Training**

---

1. Finish the OTMC internship
2. Audit the Cancer genetics course (Dr Alvaro Monteiro)
3. Continuous attending of the relevant scientific ground rounds, seminars, talks etc at Moffitt and/or USF

### **Post Doc's Proposed Goals: Publications/Presentation**

---

At least two relevant peer-reviewed publication accepted in reputable scientific journals. At least one of which is the 1st author.  
Ideally, two 1st author plus at least one non-1st author.

### **Approved Goals: Publications/Presentation**

---

At least two relevant peer-reviewed publication accepted in reputable scientific journals. At least one of which is the 1st author.  
Ideally, two 1st author plus at least one non-1st author.

### **Post Doc's Proposed Goals: Honors and Awards**

---

Travel awards where applicable.

**Approved Goals: Honors and Awards**

---

Travel awards where applicable.

**Post Doc's Proposed Goals: Grants/Proposals**

---

At least one new submission and/or re-submission as a P.I. However, my goal is at least two submissions a year (including new submissions and revisions).

**Approved Goals: Grants/Proposals**

---

At least one new submission and/or re-submission as a P.I. However, my goal is at least two submissions a year (including new submissions and revisions).

**Post Doc's Proposed Goals: Teaching**

---

Mentor undergraduate or graduate students such as SPARK / LINK.

**Approved Goals: Teaching**

---

Mentor undergraduate or graduate students such as SPARK / LINK.

**Post Doc's Proposed Goals: Service and Administration**

---

Not sure at this time, but would like to get involved.

**Approved Goals: Service and Administration**

---

Not sure at this time, but would like to get involved.

### **Post Doc's Proposed Goals: Career**

---

At least two relevant job applications a year.

### **Approved Goals: Career**

---

At least two relevant job applications a year.

### **New Goals Overall Comments (Optional):**

**Evaluator may use this area to make comments related to new goals:**

### **Post Doc Final Comments (optional):**

N/A

### **Evaluator Final Comments (optional):**

Ganna is a bright, hardworking post doctoral candidate with a focussed interest in prostate cancer disparity. She has garnered outstanding skill sets and is a versatile researcher.

Employee: Ganna Chornokur G.C. (electronic signature for the  
evaluation of Ganna Chornokur)

Date: 12/11/2012 08:51 AM



App. 15

Variable	Dr Phelan's study	Dr Park's study
<b>Case</b>	Part 7, #1: Yes, table, row "prostate cancer"	Part III, A: Yes - prostate
<b>Control</b>	Part 7, #1: No	Part III, A: No
<b>Age</b>	2012 – Part 1, #4 year only	2012 – the DOB year only (top of the 1 <sup>st</sup> page)
<b>Age at diagnosis</b> (cases only)	Part 7, #1 table, column "What was your age when you were told you have this type of cancer", row "prostate cancer".	Top of the 1 <sup>st</sup> page: [date of current diagnosis, year] – [DOB, year]
<b>Race/ethnicity:</b> - Black/Non-Hispanic or African American  - White/Non-Hispanic or Caucasian	Part 1, #1: No; and Part 1, #2: Black or African American	Part I, C: No; and Part I, D: Black or African American
	Part 1, #1: No; and Part 1, #2: White	Part I, C: No; and Part I, D: White
<b>Education:</b> - Some High school  - High school graduate  - Some college  - College graduate  - Postgraduate	Part 1, #6: less than 6 <sup>th</sup> grade; 6 <sup>th</sup> – 8 <sup>th</sup> grade; 9 <sup>th</sup> – 10 <sup>th</sup> grade;	Part I, E: grade school/junior high school; some high school
	Part 1, #6: 11 <sup>th</sup> – 12 <sup>th</sup> grade; GED or equivalent;	Part I, E: high school graduate
	Part 1, #6: some college; vocational school	Part I, E: some college/ technical/ vocational school certificate
	Part 1, #6: graduate college	Part I, E: college graduate
	Part 1, #6: postgraduate or professional school	Part I, E: post-graduate degree
<b>Relationship status:</b> - Married/living with a partner  - Widowed/ divorced/ separated/ single  - Refuse to answer	Part 1, #5: cohabitating/living together; married	Part I, A: married/living with partner
	Part 1, #5: Widowed; divorced/separated; single	Part I, A: widowed; divorced; separated; single
	Part 1, #5: Refuse to answer	Not an option
<b>Personal history of cancers</b> (other than prostate cancer): - Yes  - No	Part 7, #1: Yes, table, all rows besides prostate cancer. Please specify the cancer type	Part III, A: Yes – a. If yes, what area of the body and what was the diagnosis? Please specify the cancer type
	Part 7, #1: No	Part III, A: No
If yes to Personal history of cancers, please specify the <b>age at diagnosis</b>	Part 7, #1: table, column "what was your age when you were told that you had this type of cancer?"	Part III, A, b. In what year was your diagnosis and how old were you?
<b>Family history of cancers</b> - Yes - No/ Don't know	Part 8, #6: Yes	Part III, C: table is filled
	Part 8, #6: No; don't know	Part III, C: table is empty
If yes to family history of cancers, <b>what was the cancer site/type?</b>	Part 8, #6, table – yes to the "type of cancer" column; AND/OR Part 8, #7, table – yes to the "type of cancer" column. Please specify the type(s).	Part III, C: table, column "type (site) of cancer". Please specify the cancer type(s).
If yes to family history of cancers, <b>what blood relative(s) got it?</b>	Part 8, #6, table – column "please indicate who has had this type of cancer"; AND/OR Part 8, #7, table – column "please indicate who has had this type of cancer" Please specify the relative(s).	Part III, C: table, column containing the relatives (mother, father, sister, brother, grandmother, grandfather). Please specify the relative(s).
<b>What of these conditions do you have?</b> - Depression - Heart disease - High blood pressure - High cholesterol - Thyroid issues - Diabetes None of the above	Part 3, # 3: "yes" marked for one or more of the following: antidepressants, medication for heart condition, cholesterol lowering medication, thyroid medication, treatment of adult onset diabetes. Please document which of the above.	Part II, table: column "yes" checked for one or more of the following: diabetes, heart disease, thyroid problems, high blood pressure, depression. Please document which of the above.
	Part 3, # 3: "yes" marked for one or more	Part II, table: column "yes" checked for

	of the following: osteoporosis prevention; chemoprevention treatment; none of the above	chronic bronchitis, asthma, asbestosis, epilepsy, stroke, osteoarthritis, rheumatoid arthritis; AND/OR the column "no" checked for everything (else).
<b>Were you born in the US?</b> - Yes  - No	Part 1, #3: the box "United States" is checked Part 1, #3: the box "other" is checked	Part I, F: yes  Part I, F: no
If No to "Were you born in the US", specify the country.	Part 1, #3: the box "other" is checked. Please list the specified country.	Part I, F: a. "If no, in what country were you born?" Please list the country.
If No to "Were you born in the US", how many years have you lived in the US?	Part 1, #3: "In you were born in another country, how many years have you lived in the United States?" Please list the number.	Part I, F: b. "In what year did you come to live in the United States?" Please list the following: 2010 – [the # in the Part I, F: b.].
<b>Occupation</b>  - Refuse to answer	Part 1, #7: what is your occupation, current or former? Please list the occupation provided. Part 1, #7: "refuse to answer" box is checked	Part VIII, A: "what occupation or job have you worked at the longest?" Please list the occupation provided. Not an option
<b>Height</b>	Part 1, #8.1.: "how tall are you".	Part IV, C: "how tall are you"
<b>Weight at diagnosis</b>	Part 1, #8.2.: "how much do you currently weigh"	Part IV, B: "your current weight"
<b>BMI</b>	$\text{BMI} = (\text{weight, pounds}) * 703 / \text{height, inches}^2$	
<b>Physical activity</b> during the last year, hrs per week: - Vigorous  - Moderate  - Walking  No physical activity	Part 9, #1 [days per week] * Part 9, #2 [hours and minutes per day]  Part 9, #3 [days per week] * Part 9, #4 [hours and minutes per day] Part 9, #5 [days per week] * Part 9, #6 [hours and minutes per day] Part 9, # 1-6, options "no activities" and/or "don't know/not sure" checked.	Part IV, H: table, rows jogging or running, swimming, bicycling, aerobics, column "hours per week" Part IV, H: table, rows racket sports, team sports, dancing, column "hours per week" Part IV, H: table, row walking, column "hours per week" Part IV, H: table – no activities checked
<b>NSAIDs</b> on a regular basis - Aspirin  - Ibuprofen  - No Aspirin  - No Ibuprofen	Part 3, #1: 1 per week; 2-5 per week; 6 or more per week; 1-3 per month are checked Part 3, #2: 1 per week; 2-5 per week; 6 or more per week; 1-3 per month are checked Part 3, #1: never is checked  Part 3, #2: never is checked	Part V, B: table, "Yes" by any of the Aspirin products is checked Part V, B: table, "Yes" by any of the Ibuprofen products is checked Part V, B: table, "No" by all of the Aspirin products is checked Part V, B: table, "No" by all of the Ibuprofen products is checked
Have you ever <b>smoked</b> (chewed, snuffed etc) tobacco products? - Yes - No	Part 5, #1(b): Yes Part 5, #1(b): No	Part VI, A: Yes Part VI, A: No
If yes, are you <b>smoking</b> (chewing, snuffing etc) tobacco products now? - Yes  - No	Part 5, #1(a): Yes  Part 5, #1(a): No	Part VI, A(1): table, raw "do you still smoke (chew etc)?" – Yes to any or all tobacco products Part VI, A(1): table, raw "do you still smoke (chew etc)?" – No to <u>all</u> tobacco products
How old were you when you started <b>smoking</b> (chewing, snuffing etc) tobacco products?	Part 5, #1(c): note the age	Part VI, A(1): table, raw "how old were you when you started smoking (chewing) regularly?" – note the age(s) for any or all tobacco products
On average, <b>how many cigarettes</b> (cigars etc) did you smoke (chew etc) per day?	Part 5, #1(e): note the number	Part VI, A(1): table, raw "on average, how many cigarettes did/do you smoke per

[illegible]

App. 16

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT				1. CONTRACT ID CODE S	PAGE OF PAGES 1   11
2. AMENDMENT/MODIFICATION NO. P00001		3. EFFECTIVE DATE 29-Apr-2013		4. REQUISITION/PURCHASE REQ. NO. W91ZSQ0317N682	
6. ISSUED BY USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014		CODE W81XWH	7. ADMINISTERED BY (If other than item 6) US ARMY MEDICAL RESEARCH ACQUISITION ACT ATTN: MIRLENE ELLISON 301-619-7733 820 CHANDLER STREET FORT DETRICK MD 21702		CODE W81XWH
8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code) H. LEE MOFFITT CANCER CENTER AND RESEARCH ANN GOFF 12902 MAGNOLIA DR TAMPA FL 33612-9416				9A. AMENDMENT OF SOLICITATION NO.	
				9B. DATED (SEE ITEM 11)	
				X 10A. MOD. OF CONTRACT/ORDER NO. W81XWH-11-1-0376	
				X 10B. DATED (SEE ITEM 13) 01-Jun-2011	
CODE 1X4B9		FACILITY CODE			
11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS					
<input type="checkbox"/> The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer <input type="checkbox"/> is extended, <input type="checkbox"/> is not extended. Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.					
12. ACCOUNTING AND APPROPRIATION DATA (If required)					
13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.					
A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.					
B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).					
C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:					
X D. OTHER (Specify type of modification and authority) In accordance with USAMRAA General Terms and Conditions					
E. IMPORTANT: Contractor <input checked="" type="checkbox"/> is not, <input type="checkbox"/> is required to sign this document and return _____ copies to the issuing office.					
14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.) Modification Control Number: mellison133532 The purpose of this modification is to: 1. Extend the period of performance end date from 30 June 2013 to 30 June 2014 (Research Ends 31 May 2014) at no cost to the government in accordance with the recipient's request dated 12 April 2013, and GOR approval dated 23 April 2013. 2. Incorporate a revised SOW dated 12 April 2013, and approved by the GOR on 23 April 2013. 3. An annual technical report will be due on 30 June 2013. 4. The final technical report will be due 30 June 2014. All other terms and conditions remain unchanged					
Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.					
15A. NAME AND TITLE OF SIGNER (Type or print)			16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) JANET P KUHNS / CONTRACTING OFFICER TEL: 301-619-2827 EMAIL: janet.p.kuhns.civ@mail.mil		
15B. CONTRACTOR/OFFEROR  (Signature of person authorized to sign)		15C. DATE SIGNED		16B. UNITED STATES OF AMERICA BY <u>Janet P. Kuhns</u> (Signature of Contracting Officer)	
				16C. DATE SIGNED 26-Apr-2013	

EXCEPTION TO SF 30  
APPROVED BY OIRM 11-84

30-105-04

STANDARD FORM 30 (Rev. 10-83)  
Prescribed by GSA  
FAR (48 CFR) 53.243

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

CLIN 0001

The CLIN extended description has changed from Prostate Cancer Research Program - Health Disparity Training Award - Postdoctoral - Ph.D or M.D Period of Performance: 1 June 2011 - 30 June 2013 (Research Ends 31 May 2013) to Prostate Cancer Research Program - Health Disparity Training Award - Postdoctoral - Ph.D or M.D Period of Performance: 1 June 2011 - 30 June 2014 (Research Ends 31 May 2014).

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 01-JUN-2011 TO 30-JUN-2013	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	UIC
POP 01-JUN-2011 TO 30-JUN-2014	N/A	USA MED RESEARCH MAT CMD 1077 PATCHEL STREET BLDG 1077 FORT DETRICK MD 21702 FOB: Destination	W91ZSQ

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

A. This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's revised statement of work dated 12 April 2013, and the revised budget dated 11 May 2011 for this proposal submitted in response to the Fiscal Year 2010 (FY10) Department of Defense Prostate Cancer Research Program - Health Disparity Training Award - Postdoctoral - Ph.D or M.D Announcement, which closed May 26, 2010, are incorporated herein by reference. The Catalog of Federal Domestic Assistance Number relative to this award is CFDA 12.420.

**B. ACCEPTANCE OF AWARD:** The recipient is not required to countersign this assistance award. In case of disagreement, the recipient shall notify the Grants Officer and not assess the award any costs until such disagreement(s) is resolved.

**C. USAMRAA GENERAL TERMS AND CONDITIONS:** This assistance agreement is subject to the USAMRAA General Terms and Conditions and to any special considerations as contained in the below mentioned Section titled "Special Terms and Conditions". These USAMRAA General Terms and Conditions are incorporated by reference with the same force and effect as if they were given in full text. The full text of the USAMRAA General Terms and Conditions may be accessed electronically at <http://www.usamraa.army.mil>.

**D. SPECIAL TERMS AND CONDITIONS**

**1. TRAINING TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)**

**ANNUAL SUMMARY**

A 2-5 page annual summary in electronic format (PDF or Word file only), presenting a description of the training and research accomplishments to date, shall be submitted within 30 calendar days after the anniversary date of the award to <https://ers.amedd.army.mil>.

All reports shall have the following elements in this order:

**FRONT COVER:** Sample front cover provided at <https://mrmc.amedd.army.mil/rpindex.asp>. The Accession Document (AD) Number should remain blank.

**STANDARD FORM 298:** Sample SF 298 provided at <https://mrmc.amedd.army.mil/rpindex.asp>. The abstract in Block 13 must state the purpose, scope, major findings and be an up-to-date report of the progress in terms of results and significance. Subject terms are keywords that may have previously assigned to the proposal abstract or are keywords that may be significant to the training grant. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Please count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers should be typed: please do not hand number pages.

**TABLE OF CONTENTS:** Sample table of contents provided at <https://mrmc.amedd.army.mil/rpindex.asp>.

**INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the training grant.

**BODY:** The content of the report should address the training and research accomplishments associated with the tasks outlined in the approved Statement of Work. Any technical or unexpected difficulties encountered and/or any deviations from the original Statement of Work should be addressed. However, any changes to the original Statement of Work must be approved by the Army Grants Officer's Representative. This approval must be obtained prior to initiating any change to the original Statement of Work. Journal articles can be substituted for detailed descriptions of specific aspects of the research/training, but the original articles must be attached to the report as an appendix and appropriately referenced in the text.

**KEY RESEARCH ACCOMPLISHMENTS:** Bulleted list of key research accomplishments emanating from this training grant.

**REPORTABLE OUTCOMES:** Provide a list of reportable outcomes that have resulted from this training grant to include:

manuscripts, abstracts, presentations; patents and licenses applied for and/or issued; degrees obtained that are supported by this award; development of cell lines, tissue or serum repositories; informatics such as databases and animal models, etc.; funding applied for based on work supported by this award; employment or research opportunities applied for and/or received based on experience/training supported by this award.

**CONCLUSION:** Summarize the results to include the importance and/or implications of the completed training grant and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

**REFERENCES:** List all references pertinent to the report using a standard journal format (i.e. format used in *Science*, *Military Medicine*, etc.).

**APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Pages shall be consecutively numbered throughout the report. DO NOT RENUMBER PAGES IN THE APPENDICES.

Mark all pages of the report which contain proprietary or unpublished data that should be protected by the U.S. Government. Reports not properly marked for limitation will be distributed as Approved for Public Release. It is the responsibility of the Principal Investigator to advise the U.S. Army Medical Research and Materiel Command when restricted limitation assigned to a document can be downgraded to Approved for Public Release. DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS.

### **Manuscripts/Reprints, Abstracts**

A copy of manuscripts or subsequent reprints resulting from the research shall be submitted to the USAMRMC. An extended abstract suitable for publication in the proceedings of the applicable research program is required in relation to a DOD meeting planned during the term of this award. The extended abstract shall (1) identify the accomplishments since award and (2) follow instructions to be prepared by the USAMRMC and promulgated at a later date. The extended abstract style will be dependent on the discipline.

## **2. ADVANCE PAYMENTS AND FULL FUNDING (NOV 2000) (USAMRAA)**

a. Payments. Advance payments will be made to the recipient. Questions relative to payment issues involving Defense Finance and Accounting Service shall be directed to [USAMRAA.GREEN@AMEDD.ARMY.MIL](mailto:USAMRAA.GREEN@AMEDD.ARMY.MIL).

b. Electronic Funds Transfer. All advance payments to the recipient will be made by electronic funds transfer (EFT). The recipient shall contact the Defense Finance and Accounting System (DFAS) named on the face page of this award to make arrangements for EFT. Failure to do so may result in nonpayment.

c. If the recipient fails to perform, the Grants Officer shall notify DFAS in writing to withhold payments.

d. Advance Payment Schedule

Year One \$56,530.00

<u>Amount</u>	<u>On or About</u>
\$14,132.50	Upon execution of this award
\$14,132.50	1 September 2011
\$14,132.50	1 December 2011
\$14,132.50	1 March 2012

Year Two \$61,222.00

<u>Amount</u>	<u>On or About</u>
\$15,305.50	1 June 2012
\$15,305.50	1 September 2012
\$15,305.50	1 December 2012
\$15,305.50	1 March 2013

e. Financial Reporting Requirements:

Federal Financial Report (SF 425): Quarterly and Final Reports (For reporting individual assistance agreements)

Reporting period end dates fall on the end of the calendar quarter for quarterly reports (3/31, 6/30, 9/30, 12/31) and the end date of the assistance agreement period of performance for the final report. Reports are due 30 days after the reporting period end date for quarterly reports and 90 days after the end date of the assistance agreement for the final report.

The SF425 and instructions for completion can be obtained from <https://usamraa.army.mil>. All SF425's shall be submitted electronically to [USAMRAASF425@amedd.army.mil](mailto:USAMRAASF425@amedd.army.mil). The award number assigned by USAMRAA, which looks similar to W81XWH-XX-X-XXXX shall be included in the subject line of the electronic submission.

NOTE: The SF425 is a single form that consolidates and replaces the Federal Cash Transaction Report (SF272.SF272A) and the Financial Status Report (SF269/SF269A)

f. Interest Bearing Account. Unless exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (CMIA) (31 U.S.C. 3335), the recipient shall deposit all advance payments in an interest bearing account. Interest over the amount of \$250 per year shall be remitted annually to the Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, MD 20852. A copy of the transmittal letter stating the amount of interest remitted shall be sent to the U.S. Army Medical Research Acquisition Activity, ATTN:MCMR-AAA-E, 820 Chandler Street, Fort Detrick, MD 21702-5014.

### 3. PROHIBITION OF USE OF LABORATORY ANIMALS (JAN 2007) (USAMRAA)

**\*\* PROHIBITION – READ FURTHER FOR DETAILS \*\***

Notwithstanding any other provisions contained in this award or incorporated by reference herein, the contractor is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the US Army Medical Research and Materiel Command, Animal Care and Use Office (ACURO). The contractor will receive written approval to begin research under the applicable protocol proposed for this award from the US Army Medical Research and Materiel Command, ACURO, under separate letter. A copy of this approval will be provided to the US Army Medical Research and Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in the termination of the award.



**4. PROHIBITION OF HUMAN RESEARCH (JAN 2007) (USAMRAA)****\*\* PROHIBITION – READ FURTHER FOR DETAILS \*\***

Research under this award involving the use of human subjects, to include the use of human anatomical substances and/or human data, may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human subjects under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the contractor. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

**5. PROHIBITION OF USE OF HUMAN CADAVERS (JAN 2007) (USAMRAA)****\*\* PROHIBITION – READ FURTHER FOR DETAILS\*\***

Research under this award using human cadavers may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human cadavers under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the contractor. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

**6. MAXIMUM OBLIGATION (SEP 2006) (USAMRAA)**

The maximum obligation for support of the project will not exceed the amount specified in the award, as amended. USAMRAA does not amend assistance agreements to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits and other costs.

**7. SUPPORTING INFORMATION (APR 2008) (USAMRAA)**

Information such as subawards, consultant agreements, vendor quotes, and personnel work agreements may be required in order to support proposed costs or to determine the employment status of personnel under the assistance agreement. The Government's receipt of this information does not constitute approval or acceptance of any term or condition included therein. The terms and conditions of the assistance agreement take precedence over any term or condition included in supporting information.

2 CFR Part 170  
Requirements for Federal Funding Accountability and Transparency Act Implementation

Appendix A to Part 170--Award Term

I. Reporting Subawards and Executive Compensation

A. Reporting of first-tier subawards.

1. Applicability. Unless you are exempt as provided in paragraph D. of this award term, you must report each action that obligates \$25,000 or more in Federal funds that does not include Recovery funds (as defined in section 1512(a)(2) of the American Recovery and Reinvestment Act of 2009, Pub. L. 111-5) for a subaward to an entity (see definitions in paragraph e. of this award term).

2. Where and when to report.

i. You must report each obligating action described in paragraph a.1. of this award term to <http://www.fsrs.gov>.

ii. For subaward information, report no later than the end of the month following the month in which the obligation was made. (For example, if the obligation was made on November 7, 2010, the obligation must be reported by no later than December 31, 2010.)

3. What to report. You must report the information about each obligating action that the submission instructions posted at <http://www.fsrs.gov> specify. must report the information about each obligating action that the submission instructions posted at <http://www.fsrs.gov> specify.

B. Reporting Total Compensation of Recipient Executives.

1. Applicability and what to report. You must report total compensation for each of your five most highly compensated executives for the preceding completed fiscal year, if--

i. the total Federal funding authorized to date under this award is \$25,000 or more;

ii. in the preceding fiscal year, you received--

(A) 80 percent or more of your annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

iii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. Where and when to report. You must report executive total compensation described in paragraph b.1. of this award term:

i. As part of your registration profile at <http://www.ccr.gov>.

ii. By the end of the month following the month in which this award is made, and annually thereafter.

C. Reporting of Total Compensation of Subrecipient Executives.

1. Applicability and what to report. Unless you are exempt as provided in paragraph d. of this award term, for each first-tier subrecipient under this award, you shall report the names and total compensation of each of the subrecipient's five most highly compensated executives for the subrecipient's preceding completed fiscal year, if--

- i. in the subrecipient's preceding fiscal year, the subrecipient received--
  - (A) 80 percent or more of its annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and
  - (B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts), and Federal financial assistance subject to the Transparency Act (and subawards); and
- ii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. Where and when to report. You must report subrecipient executive total compensation described in paragraph c.1. of this award term:

- i. To the recipient.
- ii. By the end of the month following the month during which you make the subaward. For example, if a subaward is obligated on any date during the month of October of a given year (i.e., between October 1 and 31), you must report any required compensation information of the subrecipient by November 30 of that year.

D. Exemptions. If, in the previous tax year, you had gross income, from all sources, under \$300,000, you are exempt from the requirements to report:

- i. Subawards, and
- ii. The total compensation of the five most highly compensated executives of any subrecipient.

E. Definitions. For purposes of this award term:

- 1. Entity means all of the following, as defined in 2 CFR part 25:
  - i. A Governmental organization, which is a State, local government, or Indian tribe;
  - ii. A foreign public entity;
  - iii. A domestic or foreign nonprofit organization;
  - iv. A domestic or foreign for-profit organization;
  - v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.
- 2. Executive means officers, managing partners, or any other employees in management positions.
- 3. Subaward:
  - i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.
  - ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ---- .210 of the attachment to OMB Circular A-133, "Audits of States, Local Governments, and Non-Profit Organizations").
  - iii. A subaward may be provided through any legal agreement, including an agreement that you or a subrecipient considers a contract.

4. Subrecipient means an entity that:

- i. Receives a subaward from you (the recipient) under this award; and
- ii. Is accountable to you for the use of the Federal funds provided by the subaward.

5. Total compensation means the cash and noncash dollar value earned by the executive during the recipient's or subrecipient's preceding fiscal year and includes the following (for more information see 17 CFR 229.402(c)(2)):

- i. Salary and bonus.
- ii. Awards of stock, stock options, and stock appreciation rights. Use the dollar amount recognized for financial statement reporting purposes with respect to the fiscal year in accordance with the Statement of Financial Accounting Standards No. 123 (Revised 2004) (FAS 123R), Shared Based Payments.
- iii. Earnings for services under non-equity incentive plans. This does not include group life, health, hospitalization or medical reimbursement plans that do not discriminate in favor of executives, and are available generally to all salaried employees.
- iv. Change in pension value. This is the change in present value of defined benefit and actuarial pension plans.
- v. Above-market earnings on deferred compensation which is not tax-qualified.
- vi. Other compensation, if the aggregate value of all such other compensation (e.g. severance, termination payments, value of life insurance paid on behalf of the employee, perquisites or property) for the executive exceeds \$10,000.

*End of clause*

## 2 CFR Part 25

## Financial Assistance Use of Universal Identifier and Central Contractor Registration

## Appendix A to Part 25--Award Term

## I. Central Contractor Registration and Universal Identifier Requirements

A. Requirement for Central Contractor Registration (CCR). Unless you are exempted from this requirement under 2 CFR 25.110, you as the recipient must maintain the currency of your information in the CCR until you submit the final financial report required under this award or receive the final payment, whichever is later. This requires that you review and update the information at least annually after the initial registration, and more frequently if required by changes in your information or another award term.

B. Requirement for Data Universal Numbering System (DUNS) Numbers. If you are authorized to make subawards under this award, you:

1. Must notify potential subrecipients that no entity (see definition in paragraph C of this award term) may receive a subaward from you unless the entity has provided its DUNS number to you.
2. May not make a subaward to an entity unless the entity has provided its DUNS number to you.

C. Definitions. For purposes of this award term:

1. Central Contractor Registration (CCR) means the Federal repository into which an entity must provide information required for the conduct of business as a recipient. Additional information about registration procedures may be found at the CCR Internet site (currently at <http://www.ccr.gov>).

2. Data Universal Numbering System (DUNS) number means the nine-digit number established and assigned by Dun and Bradstreet, Inc. (D&B) to uniquely identify business entities. A DUNS number may be obtained from D&B by telephone (currently 866-705-5711) or the Internet (currently at <http://fedgov.dnb.com/webform>).

3. Entity, as it is used in this award term, means all of the following, as defined at 2 CFR part 25, subpart C:

- a. A Governmental organization, which is a State, local government, or Indian Tribe;
- b. A foreign public entity;
- c. A domestic or foreign nonprofit organization;
- d. A domestic or foreign for-profit organization; and
- e. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

4. Subaward:

a. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.

b. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ---.210 of the attachment to OMB Circular A-133, "Audits of States, Local Governments, and Non-Profit Organizations").

c. A subaward may be provided through any legal agreement, including an agreement that you consider a contract.

5. Subrecipient means an entity that:

- a. Receives a subaward from you under this award; and

- b. Is accountable to you for the use of the Federal funds provided by the subaward.

*End of Clause*

(End of Summary of Changes)